THE EFFECTS OF DIETARY LIPIDS AND ANTIOXIDANTS ON GROWTH PERFORMANCE, MEAT QUALITY AND MOISTURE LOSS OF THE SOUTH AFRICAN ABALONE, *HALIOTIS MIDAE*

by

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DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: April 2014

SUMMARY

Abalone has become a high valued commodity globally, with South Africa being one of the largest producers. *Haliotis midae* is the most important aquaculture species produced in South Africa, and is exported to the Eastern markets in a variety of forms, with live export and canned abalone being the two most important products. To meet the high international demand, abalone farmers need to remain competitive by optimizing growth rate, increase water absorption, and minimising the stress and moisture loss experienced by abalone during the live export period. Stress and moisture loss experienced during export can potentially contribute to a decrease in live weight and meat quality, which in turn will result in lower income generated, which will impact negatively on the cost-efficient production of abalone.

No literature is available on reducing moisture loss in live abalone during export. Other factors such as diet composition (which can affect the growth rate of abalone), animal health condition (assessed by weight of the abalone per unit shell length), and meat composition and quality, can also affect the cost-efficient production of abalone. The aim of this study was therefore to determine the amount of moisture loss experienced by adult live abalone that have reached an export size, and to formulate a complete balanced diet that will minimize the moisture loss during export. Aspects that were investigated included a) the effect of diet on the growth rate of abalone, b) the effect of diet composition on moisture loss during live export of abalone, c) the effect of diet on the extent of *post mortem* and post cooking moisture loss, and d) the effect of diet composition on proximate and chemical composition of abalone meat.

This study evaluated the effect of ten diets that differed in terms of their Vitamin E, polyunsaturated fatty acid (PUFA), chromium, and green rooibos content, The diets consisted of Control 1 (Abfeed®), Control 2 (NutroScience), LN (low PUFA with no additives), LM (low PUFA with vitamin E (mixed tocopherols)), LR (low PUFA with green rooibos) and LCr (low PUFA with chromium), HN (high PUFA with no additives), HM (high PUFA with vitamin E (mixed tocopherols)), HR (high PUFA with green rooibos), and HCr (high PUFA with chromium). Sunflower oil was used to formulate the high PUFA treatments, whilst rendered beef fat (tallow) was used to formulate the low PUFA treatments. Animals (n=25) from each treatment was sampled monthly to determine the effect of the different treatments on the growth performance (measured as weight and length gain, average daily gain and specific growth rate in terms of weight and length, feed conversion ratio, and condition factor).

Proximate and chemical analyses were performed on the abalone meat *post mortem* to determine the effect of diet composition on moisture loss, water retention, and drip loss. Varying results were obtained for the high and low PUFA diets with added antioxidants, and it was suggested that PUFA levels and antioxidants did not play a significant role in improving the overall growth rate and meat composition of abalone. However, there was a trend for improved growth performance when the control NutroScience diet was fed,

indicating that the diet can potentially be considered as a cheaper alternative feed for abalone growth performance. Further research, however, is required in this regard before it can be propagated as such.

On completion of the feeding trial, a standard 40 hour export journey for live abalone was simulated under laboratory conditions to determine moisture loss during live export. The results from this experiment indicated that, even though not significant, PUFA and antioxidants tend to play an important role in ensuring the retention of body water, consequently reducing moisture loss. Overall, abalone fed the AquaNutro diet lost the least weight during the live export simulation, which differed significantly when compared to the weight loss experienced by abalone fed the Abfeed® diet. Abalone that gained the most moisture during the purging period (i.e. period where animals are starved to clean out their intestines) tended to lose the least total moisture after transport and cooking, respectively. These findings indicated a potential correlation between the absorption rate of water during purging, and moisture retention during handling, transport and cooking of abalone. Further studies are required to better understand the water loss and retention dynamics in live abalone during transport, and how this affects abalone meat quality, water absorption during purging and moisture retention during handling, transport and cooking.

OPSOMMING

Perlemoen is 'n hoë waarde kommoditeit wêreldwyd, met Suid-Afrika as een van die grootste produsente. *Haliotis midae* is die belangrikste akwakultuur spesie wat in Suid-Afrika geproduseer en na die Oosterse markte as veral ’n lewende en geblikte produk uitgevoer word. Om te voldoen aan die groot internasionale aanvraag, moet perlemoen produsente so kompeterend as moontlik bly deur die groeitempo van die diere te optimaliseer, waterabsorpsie tydens vervoer, asook die stres en vogverlies wat lewende diere tydens uitvoer ondervind, te beperk. Laasgenoemde kan potensieel tot ‘n afname in lewende gewig en vleiskwaliteit bydra, wat die winsgewendheid van perlemoen produksiesisteme negatief kan beïnvloed.

Geen literatuur is beskikbaar oor die beperking van vogverlies tydens uitvoer nie. Ander faktore soos dieet samestelling (wat die groeitempo van perlemoen kan beïnvloed), die gesondheid status (bepaal deur gewig van die perlemoen per eenheid dop lengte) en vleis samestelling en kwaliteit (waarvan vogverlies ’n groot komponent is) kan almal ’n invloed op die winsgewendheid van perlemoen produksie hê. Die doel van hierdie studie was dus om die mate van vogverlies ondervind deur volwasse perlemoen wat gereed is vir die lewende uitvoermark te bepaal, asook om ’n volledig gebalanseerde dieet te formuleer wat vogverlies tydens uitvoer sal beperk. Aspekte wat ondersoek is, het ingesluit a) die effek van dieet samestelling op die groeitempo van perlemoen, b) die effek van dieet samestelling op die mate van vogverlies ondervind in lewende perlemoen tydens uitvoer, c) die effek van dieet op die hoeveelheid drupverlies in perlemoen vleis ondervind post-mortem en d) die potensiële invloed van dieet op die proksimale en chemiese samestelling van perlemoen vleis.

Die studie het die effek van tien diëte, wat ten opsigte van Vitamien E-, poli-onversadigde vetsuur (PUFA)-, chroom- en groen rooibosteevlakke verskil het, op perlemoen groei, water retensie en vleiskwaliteit ondersoek. Die diëte het bestaan uit Kontrole dieet 1 (Abfeed ®), Kontrole dieet 2 (NutroScience), LN (lae PUFA met geen bymiddels), LM (lae PUFA met vitamien E (gemengde tokoferole)), LR (lae PUFA met groen rooibos) en LCr (lae PUFA met chroom), HN (hoë PUFA met geen bymiddels), HM (hoë PUFA met vitamien E (gemengde tokoferole)), HR (hoë PUFA met groen rooibostee) en HCr (hoë PUFA met chroom). Sonneblomolie is gebruik om die hoë PUFA behandelings te formuleer, terwyl gereduseerde gefiltreerde beesvet gebruik is vir die formulering van die lae PUFA diëte. Diere (n=25) van elke behandeling is maandeliks gemonster om die effek van die verskillende behandelings op die groeiprestasie (gemeet aan toename in gewig en lengte, gemiddelde daaglikse toename en spesifieke groeitempo in terme van gewig en lengte, voeromsetverhouding en liggaamskondisie faktor) te bepaal.

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Perlemoen vleismonsters is post-mortem met behulp van proksimale en chemiese analises ontleed om die invloed van die onderskeie diëte op vogverlies, water retensie en drupverlies te bepaal. Variërende resultate is verkry vir die onderskeie diëte. Resultate in dié studie het aangedui dat PUFA vlakke en antioksidante nie

'n beduidende rol in die groeitempo en samestelling van perlemoenvleis gespeel het nie. Daar was egter 'n neiging vir ’n versnelde groeitempo ondervind in diere wat die NutroScience dieet ontvang het, wat dui op die potensiaal van die dieet om as 'n goedkoper alternatief vir kommersiële produksie van perlemoen gebruik te kan word. Verdere navorsing word egter benodig in dié verband voor dit so gepropageer kan word.

Na afloop van die dieetkomponent van die studie, is 'n standaard 40 uur uitvoerperiode vir lewende perlemoen nageboots onder laboratorium toestande om die omvang van vogverlies tydens uitvoer te bepaal. Die resultate van hierdie eksperiment het aangedui dat PUFA en antioksidante neig om 'n belangrike rol te speel in die behoud van liggaamswater, wat bydra tot ’n vermindering in vogverlies tydens uitvoer. Diere wat die AquaNutro dieet ontvang het, het die minste gewig gedurende die gesimuleerde uitvoer reis verloor, wanneer dit met die Abfeed® dieet vergelyk is. Diere wat die hoogste vogopname tydens die suiweringsproses (d.i. tydperk waar diere uitgehonger word om die spysverteringsisteem skoon te maak) getoon het, het geneig om die minste totale vog na die vervoer en kookproses te verloor. Hierdie bevindinge dui op ’n potensiële korrelasie tussen die opname van water tydens die suiweringsproses en die mate van vogretensie tydens hantering, vervoer en kookprosesse. Verdere studies is nodig om die dinamika van vog verlies en –retensie tydens vervoer van lewende perlemoen, asook die invloed op vleiskwaliteit te verstaan.

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LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
µL	Micro litres
ADG_L	Average Daily Gain (Length)
ADG_W	Average Daily Gain (Weight)
Anon	Anonymous
ANOVA	Analysis of Variance
AOAC	Association of Analytical Communities
Avg	Average
BHT	Butylated Hydroxytoluene
CF_I	Britz's Condition Factor (Initial)
CF_F	Britz's Condition Factor (Final)
EDTA	Ethylenediaminetetraacetic acid
FAME	Extraction of the Fatty Acid Methyl Esters
FCR	Feed Conversion Ratio
g	Gram
h	Hour
H_2O	Water
HPLC	High-performance liquid chromatography
HCr	High Polyunsaturated Fatty Acids with Chromium
HN	High Polyunsaturated Fatty Acids with No Additives
HM	High Polyunsaturated Fatty Acids with Vitamin E
HR	High Polyunsaturated Fatty Acids with Rooibos
L_I	Shell Length (Initial)
L_F	Shell Length (Final)
LSD	Least Significant Differences
LCr	Low Polyunsaturated Fatty Acids with Chromium
LN	Low Polyunsaturated Fatty Acids with No Additives
LM	Low Polyunsaturated Fatty Acids with Vitamin E
LR	Low Polyunsaturated Fatty Acids with Rooibos
mg	Milligram
ml	Millilitres
mm	Millimetres
MUFA	Monounsaturated Fatty Acids
NaCl	Sodium chloride

n-3	Omega 3 fatty acids
n-6	Omega 6 fatty acids
n-6:n-3	Omega 6 to omega 3 ratio
pH	Power of Hydrogen
PUFA	Polyunsaturated Fatty Acids
PUFA:SFA	Polyunsaturated to Saturated Fatty Acid Ratio
SD	Standard Deviation
S.E.M.	Standard Error of the Mean
SFA	Saturated Fatty Acids
SGR_L	Specific Growth Rate (Shell length)
SGR_W	Specific Growth Rate (Bodyweight)
v/v	Volume to Volume Ratio
W_I	Bodyweight (Initial)
W_F	Bodyweight (Final)

SPECIFIC DEFINITIONS AS DEFINED IN THIS THESIS

Live Export Moisture Loss	Moisture loss experienced by abalone during a simulated 40h live export period
Transport Moisture Loss`	Moisture loss experienced by abalone during a 1.5h live transport period to the processing plant
Post Mortem Moisture Loss	Moisture loss experienced by abalone after they have been slaughtered
Post Cooking Moisture Loss	Moisture loss experienced by abalone after a 5 minute cooking process
Purging	Process where animals are starved for 5 days to clean out their intestines

NOTES

The language and style used in this thesis are in accordance with the requirements of the *South African Journal of Animal Science*. This dissertation represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

Results from this study have been presented at the following symposium:

9th Biannual Aquaculture Conference of the Aquaculture Association of Southern Africa (AASA), 8-11 September 2009, Swakopmund, Namibia.

CONTENT

CHAPTER 1

GENERAL INTRODUCTION

Abalone (*Haliotis* species) is a commercially exploited marine snail (Godfrey 2003) which has a live market value of between $35 and $45 per kg (Anon, 2013a). Its global distribution is wide and varied and it inhabits rocky reefs in the intertidal and sub-tidal zones at depths of up to 50m, preferring temperate waters of 12 to 21 °C (Elliott 2000; Franchini *et al*., 2011; van Schalkwyk, 2011). However, certain species are found as far south as New Zealand (*H. iris, H. australis* and *H. virginea*) and as far north as Alaska *H. kamtschatkana* (Lindberg, 1992; Wood, 1993; Van der Merwe, 2009). Within South Africa, *H. midae* is widespread and ranges from just north of Port St John's on the east coast to St Helena Bay on the west coast (Fig. 1.1) (Lindberg, 1992; Britz *et al*., 1997; Branch *et al.*, 2002; Van der Merwe, 2009).

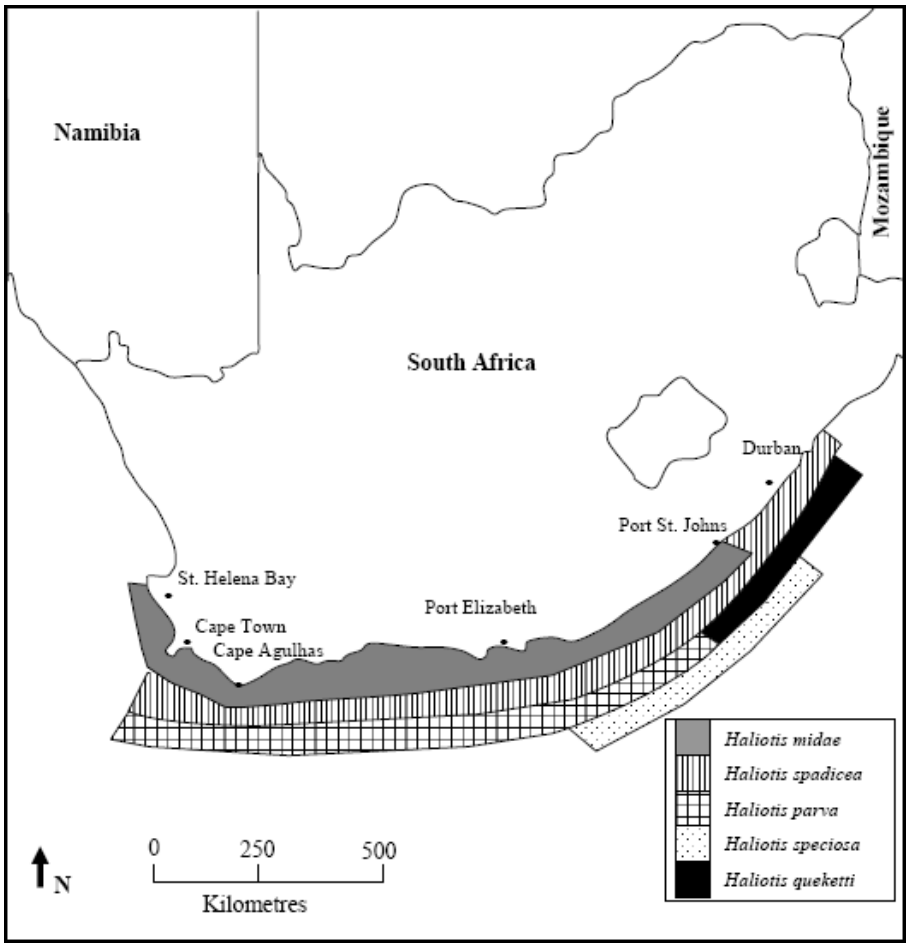

Figure 1.1 Distribution of *Haliotis* spp. along the South African coastline (Lindberg, 1992). Note that the distribution of *H. pustulata* is not represented.

Due to the sessile nature of abalone, individual distribution does not vary widely and individuals normally remain within the area in which they were seeded. Local movement is generally dictated by the availability of appropriate substrate and preferred food items (Godfrey, 2003) which include macro algae species such as *Ecklonia maxima, Laminaria palli* and *Gracillariod* species (Newman, 1969; Iyer *et al.,* 2005).

Over the last four decades global catches of abalone have decreased throughout its global distribution from almost 20 000 tonnes in the 1970's to 8 846 tonnes in 2006 (Anon, 2013b). This dramatic decrease in the natural population is a result of exploitation (both recreationally and commercially), poaching, predation, diseases, pollution and insufficient wild stock management (Tarr 1993; White, 1995; Godfrey 2003; Stevens, 2003). The reduction in the natural stocks has resulted in an increase in the artificial production of abalone for the international market as observed in Figure 1.2 (Britz, 1996b; Anon, 2013b). In 2011, a total estimate of 85 000 tonnes of abalone was produced globally (Anon, 2013b). The majority of abalone aquaculture occurs in Asia (Anon, 2014a), while farming is extensive in many other countries including Australia, USA, Mexico, New Zealand, Ireland, Iceland and South Africa (Troell *et al*., 2006).

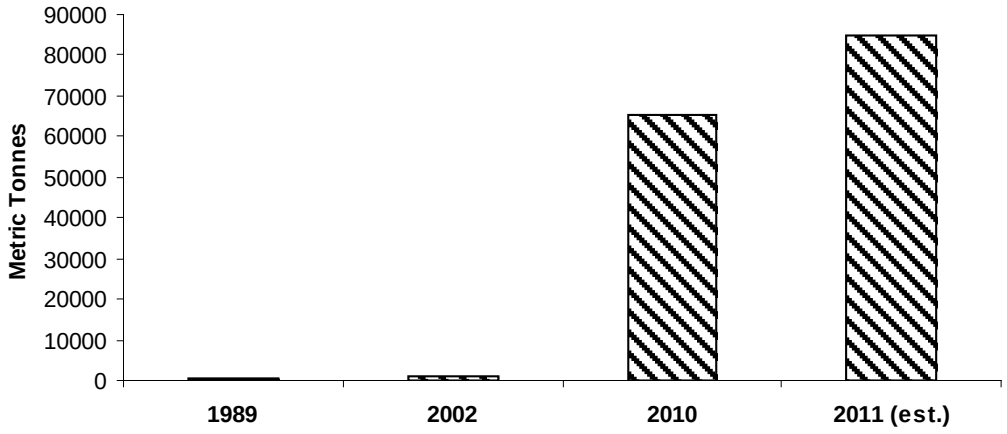

Figure 1.2 Aquaculture production of abalone globally (Anon, 2013b)

A total of six indigenous species of abalone (*H. pustulata; H. spadicea; H. queketti; H. parvum; H. speciosa;* and *H. midae)* occur along the South African coastline but only *H. midae* (locally known as "perlemoen") were commercially exploited and are currently farmed (Sales & Britz, 2001). The legal harvest of the indigenous abalone (*H. midae*) has been closed since 2008 due to the decimation of natural stocks (Raemaekers *et al*., 2011); however limited controlled fishing has been allowed since November 2011 in demarcated areas (Anon, 2014b). The general closure has increased interest in the viability of aquaculture, ranching and stock enhancement projects along the South African coast. Since 2008 South Africa has grown to become the third largest producer of abalone in the world producing 1 036 tonnes in 2011 with a value of R357 million, representing 94% of the value of the entire marine aquaculture sector within South Africa

(Anon, 2013b; DAFF, 2012). Although abalone aquaculture production is steadily increasing in South Africa (Fig 1.3) there can be significant loss in revenue due to inadequate handling, storage and transport.

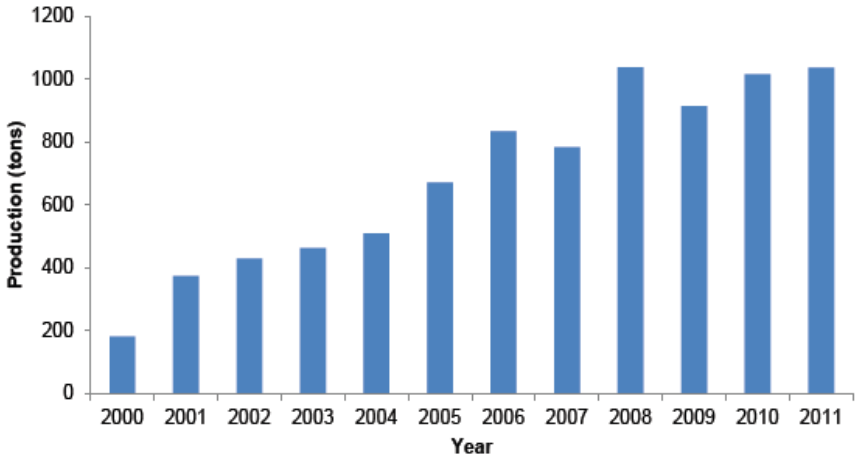

Figure 1.3 Aquaculture production of abalone in South Africa (DAFF, 2012)

During the live export process animals may lose up to 15% of their body weight due to the loss of moisture which is usually referred to as water loss, drip loss or mass loss (Vosloo & Vosloo, 2006). This can result in major financial/income loss as farmers are paid on landed rather than harvested weight. In addition, post-mortem moisture loss occurs subsequent to slaughtering due to the *rigor mortis* process (Sales *et al.*, 1999). Moisture loss is not unique to abalone and occurs in a number of marine gastropods (mussels, periwinkles and scallops) and other farmed land animals; however no consensus has been reached regarding optimal practice in relation to abalone. Aerial exposure can also affect the water holding capacity of abalone which can lose moisture due to the high stress experienced when out of their natural aquatic environment (Vosloo & Vosloo, 2006; van Schalkwyk, 2011). The addition of antioxidants in abalone diet have been shown to prevent peroxidation by reducing the propagation reaction chain length, which in turn can increase the water holding ability of the cell membrane (Gordon, 1990). Further investigation into the effects of antioxidants on abalone cell water holding capacity is therefore warranted.

The significant loss of weight and consequently revenue is a real issue in the abalone export trade and requires scientific examination which will yield recommendations on best practice. In addition, moisture loss may also affect nutritional composition and sensory perception which can further affect both product price and consumer choice. Therefore the overarching aim of this study is to investigate the extent of moisture loss in abalone and identify suitable ways of decreasing moisture loss through feed manipulation.

This study was facilitated through a number of objectives:

1. Identify the effect of diet on the growth rate.
2. Establish if diet effects moisture loss during the live export of abalone.

3. Investigate the effect of diet on the extent of local live transport, post-mortem and post cooking moisture loss.
4. Identify possible effects of abalone diet on chemical and proximate composition of the abalone muscle/meat.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

South Africa is regarded as one of the leading producers of farmed abalone in the world, with a production of more than 1 000 tons per year (DAFF, 2012; Anon, 2014). Abalone farming contributes just over half of the total marine aquaculture production every year and over 90% of the total value of the South African marine aquaculture industry (DAFF, 2012).

Due to the high international market demand and competitive nature of the industry, it is important to achieve optimal growth rate, low FCRs and produce abalone of high quality in the shortest time possible. Such practice can lead to lower production cost and an increase in turnover, subsequently increasing overall profitability. Abalone growth rate and FCR can be significantly affected by diet and feeding strategies (Leighton, 1974; Britz, 1996a & 1996b; Fleming *et al*., 1996; Guzmán & Viana, 1998; Shpigel *et al*., 1999; Boarder & Shpigel, 2001; Bautista-Teruel *et al*., 2003; Gomez-Montes *et al*., 2003; Naidoo *et al*., 2006; Ten Doeschate & Coyne, 2008); therefore the development of an optimal nutritional diet can significantly enhance abalone growth and overall harvest.

Although the requirements for certain nutrients such as protein (Britz, 1996a), lipid (Mai *et al*., 1995), vitamins and minerals (Coote *et al*., 1996) have been identified, there remains a lack of information regarding the importance of PUFA and added antioxidants. Lipids are considered the most important sources of energy in animal muscle (Durazo-Beltrán *et al*., 2003) and are vital in abalone nutrition (Nelson *et al*., 2002) whilst antioxidants are crucial in preventing deterioration of food quality by mitigating the oxidation process which can lead to moisture loss during different stages of processing. Over 60% of the abalone production in South Africa is exported live. During this export period, the animals undergo extreme stress and lose up to 15% of the body mass through water or moisture loss (Vosloo & Vosloo, 2006), which in turn decreases profitability.

Therefore, the aim of this study is to identify the effect of PUFA and antioxidant additives on abalone growth performance and moisture loss during live export, local transport as well as *post mortem* and post cooking. It is first necessary to understand the build and function of the cell and its membrane and the effects of PUFA and antioxidants on a physiological level.

2.2 Cells and Cell Membranes

The cell membrane, also called plasma membrane, surrounds cells at their surfaces (Fig 2.1). Cell membranes are thin, complex structures that are lipid-based which surrounds the cytoplasm, including the cell organelles and cytosol, as well as the nucleus (Randall *et al.*, 1998; Silverthorn *et al*., 2007). The internal cell organelles and nucleus have their own surface membranes, called the intracellular membranes. The obvious function of the cell membrane is its enclosing feature, but it is also its most critical function. The membrane regulates molecular movement, with the help of various metabolic mechanisms, between the

orderly interior of the cell and the more disorderly, potentially disruptive external environment (Raven & Johnson, 1995; Hill *et al.,* 2004).

According to Randall *et al.* (1998), all biological membranes, including the intracellular membranes of eukaryotic cells, have essentially the same structure, namely lipid and protein molecules kept together by non-covalent interactions. The lipid molecules are arranged in a continuous double layer, called the lipid bilayer, and is impermeable to most water-soluble molecules.

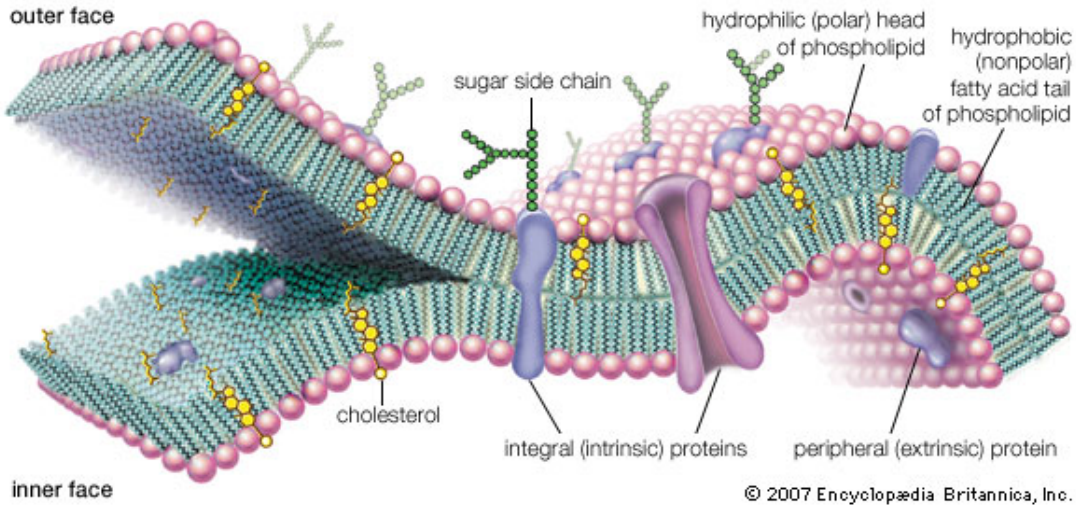

Figure 2.1 Illustration of a cell membrane (Anon, 2013a)

Lipids provide the primary structure of the membrane and are better known as phospholipids (Fig 2.2). They comprise about half the mass of cell membranes in animal cells, the rest being essential proteins. Integral proteins embedded in the membrane play more specialized roles such as transporting molecules through the membrane, catalysing reactions, and transducing chemical signals (Raven & Johnson, 1995; Randall et al., 1998). According to Randall et al. (1998) the three primary types of lipids in cell membranes are phosphoglycerides which are characterized by a glycerol backbone, sphingolipids, which have backbones made of sphingosine bases and sterols, such as cholesterol which are nonpolar and only slightly soluble in water.

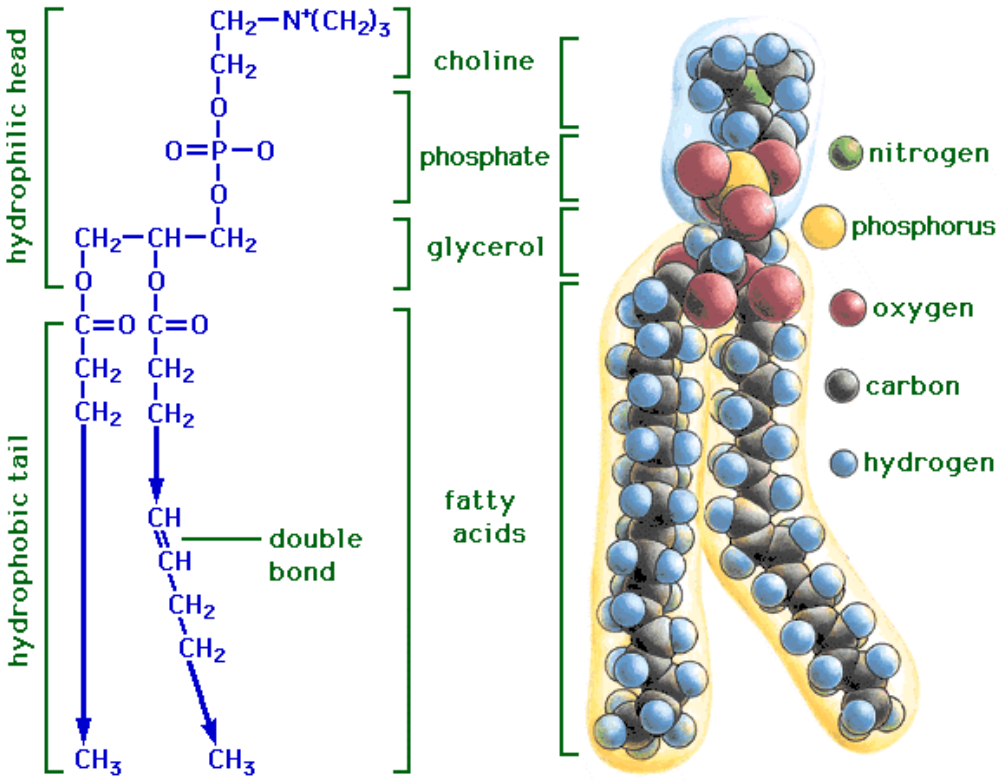

Figure 2.2 General structure of a Phospholipid (Anon, 2013b)

A membrane phospholipid consists of a polar head and two nonpolar tails, also known as a hydrophilic (water-soluble) head and hydrophobic (water-insoluble) tail (Raven & Johnson, 1995; Randall et al., 1998; Hill et al., 2004; Silverthorn et al., 2007). The hydrophilic head is made up of the phosphate group, which forms a positive charge region, bonds to another group and forms a region of negative charge, such as choline. The hydrophobic tail contains a long-chain hydrocarbon derived from a fatty acid. The double nature of these amphipathic membrane lipids, with their hydrophilic heads and hydrophobic tails, is vital to the organization of biological membranes (Hill et al., 2004). According to Randall et al. (1998), the phospholipid's polar heads seek water while their nonpolar tails seek one another, being equally attracted by van der Waals forces. The molecules are therefore perfectly suited to form a border between a non-aqueous lipid environment within the membrane itself and the aqueous intra- and extracellular phases in contact with the inner and outer membrane surfaces (Raven & Johnson, 1995; Silverthorn et al., 2007).

2.3 The effect of Fatty Acids and Cholesterol on cell membrane structure / fluidity

Phospholipid molecules are not covalently bound to each other and can move relative to one another; therefore they are fluid in a cell membrane and are able to move freely by diffusion within each membrane leaflet. The length differences of the two fatty acid chains as well as the differences in their composition

influence lipid packing and thus the fluidity, causing slight differences in lipid bilayer characteristics (Hill *et al.,* 2004).

The quantity of double bonds in the hydrocarbons is responsible for defining the chemical saturation that helps determine membrane fluidity. A hydrocarbon is saturated when it contains no double bonds and is unsaturated when one or more double bonds are present (Hill *et al.,* 2004). Most of the animal cell membrane phospholipid acyl chains contain either saturated (C – C bonds) or monounsaturated (one C = C bond) hydrocarbon polymers (Olbrich *et al*. 2000). However, membranes rich in polyunsaturated (multiple C = C bonds) lipids are present in certain animal tissue such as the brain; which can differ significantly in length. The part of the tail where a double bond occurs may cause a bend, which prevent tight, crystal-like packing of the tails in the hydrophobic interior of the membrane. This disruption of tight packing helps keep the phospholipid molecules free to move and results in a membrane with more fluidity. Olbrich *et al.* (2000) showed that the lipid bilayers containing more polyunsaturated fatty acids are more permeable and weaker than the typical monounsaturated lipid bilayer. Furthermore, a fatty acid chain containing more than two alternating *cis*-double bonds will increase membrane permeability (Olbrich *et al.,* 2000).

In addition to chemical composition, temperature also affects the fluidity of membranes. The lower the temperature drop, the stiffer the phospholipids within the cell membrane become. As mentioned earlier cell membranes contain other classes of lipids such as sterols. The main membrane sterols are cholesterol and cholesterol esters which are mainly nonpolar and only slightly soluble in water. In a water solution they form complexes with proteins that are far more water-soluble than the sterols alone. To ensure fluidity of eukaryotic cell membranes at low temperatures, cholesterol plays an important role in governing the membrane characteristic (Randall *et al.,* 1998). When cholesterol is present, it binds weakly to adjacent phospholipids, and increases the viscosity of the hydrocarbon core of the membrane, but at the same time making lipid bilayers significantly less fluid, but stronger. If too much cholesterol is present in the cell membranes, it will cause the membrane to loss flexibility (Randall *et al.,* 1998).

2.4 Fatty Acid Metabolism

Fatty acids (FAs) are covalently bonded carbon atom chains with or without double bonds. The majority of FAs have zero to four double bonds. Palmitic acid (16:0) is the most common saturated FA in adipose tissue, while oleic acid (18:1), the most widespread FA. The so-called essential fatty acids include the polyunsaturated fatty acids (PUFAs), linoleic acid (18:2, omega-6), linolenic acid (18:3, omega-3), and arachidonic acid (20:4, omega-6) (Ruckebusch *et al.,* 1991). FAs are essential nutrients because they (double bonds in the omega-3 or omega-6 position) cannot be synthesize within the body and must be obtained through diet.

Hill *et al*. (2004) and Silverthorn *et al*. (2007) noted that an important source of energy for many organisms is fatty acids which are usually ingested as triacylglycerols. Tricylglycerols are oils and are insoluble in the aqueous surroundings of the intestine, therefore emulsification of the dietary lipids is vital and is done by means of the bile salts which are produced in the liver and secreted from the gallbladder. The secretion of pancreatic lipases (lipase and phospholipase A_2), by the pancreas into the intestine, degrades

the emulsified fats to produce free fatty acids and a mixture of mono- and diacylglycerols from dietary triacylglycerols (Randall *et al.,*1998; Hill *et al*., 2004).

According to Silverthorn *et al*. (2007), after the absorption of pancreatic lipase products (free fatty acids and a mixture of mono- and diacylglycerols) by the intestinal mucosal cells, the resynthesizes of triacylglycerols takes place. The triacylglycerols are then turned into lipoprotein complexes known as chylomicrons through solubilisation. The chylomicron comprises of lipid droplets enclosed by polar lipids and proteins and are released from the intestine into the blood via the lymph system to be delivered to various tissues for storage or production of energy via oxidation. Triacylglycerols produced in the liver are released directly into the blood by way of packing them into very-low-density lipoproteins (VLDLs).

Raven & Johnson (1995) and Silverthorn *et al*. (2007) noted that glycerol is the backbone for triacylglycerols and that three fatty acids have been esterified to it. Prior to oxidation by NAD^+ and FAD, fatty acids need to be hydrolysed to become extremely reduced molecules (Fig 2.3). Thereafter, ATP is produced by passing the reducing potential through the electron transport chain. Acetyl CoA also gets produced by fatty acid oxidation and enters the citric acid cycle (Krebs cycle) to produce more NADH and $FADH_2$.

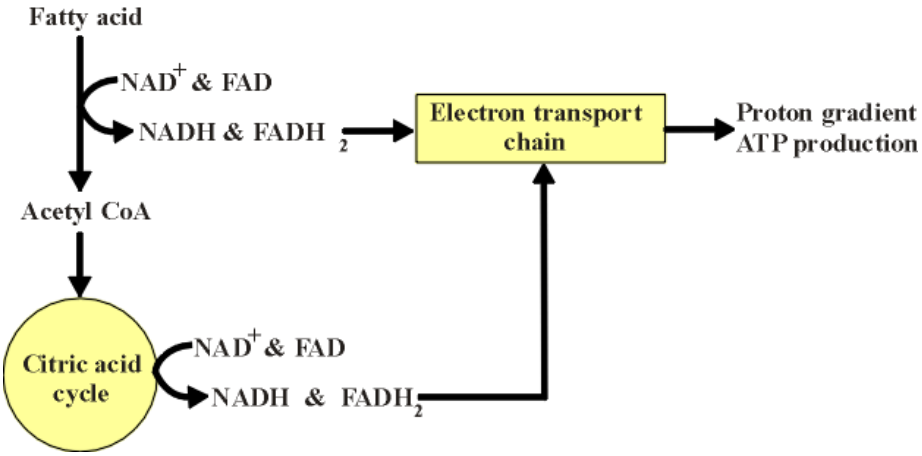

Figure 2.3 Fatty acid oxidation process (Anon, 2013c)

According to Raven & Johnson (1995) and Horton *et al*. (2006), fatty acids undertake a three step oxidation process, i.e. the activation on the outer membrane of the mitochondrial, Acyl CoA transport into the mitochondria, and to conclude the oxidation of Acyl CoA.

Step 1: Activation on the outer mitochondrial membrane

Prior to oxidation, fatty acids need to be activated by attaching CoA (Fig 2.4) which happens in the cytoplasm side of the outer mitochondrial membrane (cytosol), and are then catalysed by acyl CoA synthetase. Hydrolyzation of ATP into AMP and pyrophosphate (PPi) occurs, and subsequently pyrophosphatase hydrolyses PPi to inorganic phosphate (Pi) by consuming a second "high energy" phosphate bond. This

reaction is driven by the hydrolysis of these two high energy bonds, of which the energy change is so large that this response is irreparable. In other words, two ATP is necessary for the activation step of fatty acids.

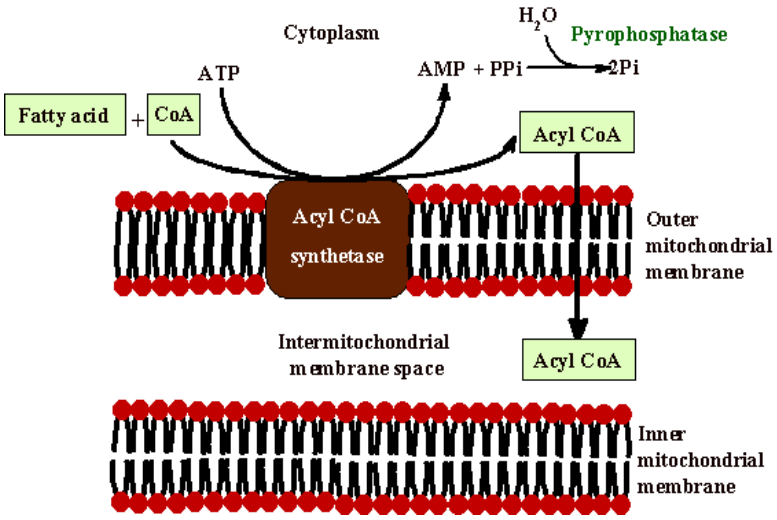

Figure 2.4 Activation of fatty acids on the outer mitochondrial membrane before oxidation (Anon, 2013c)

Step 2: Transport of Acyl CoA into mitochondria

After the activation process oxidation occurs inside of the mitochondria. Due to the impermeable nature of the inner mitochondrial membrane to long chain acyl CoA molecules, a transport system is crucial. The acyl part of acyl CoA is a long chain fatty acid, and is transformed by carnitine acyltransferase I to acylcarnitine, which occurs in the intermitochondrial membrane space (Fig 2.5).

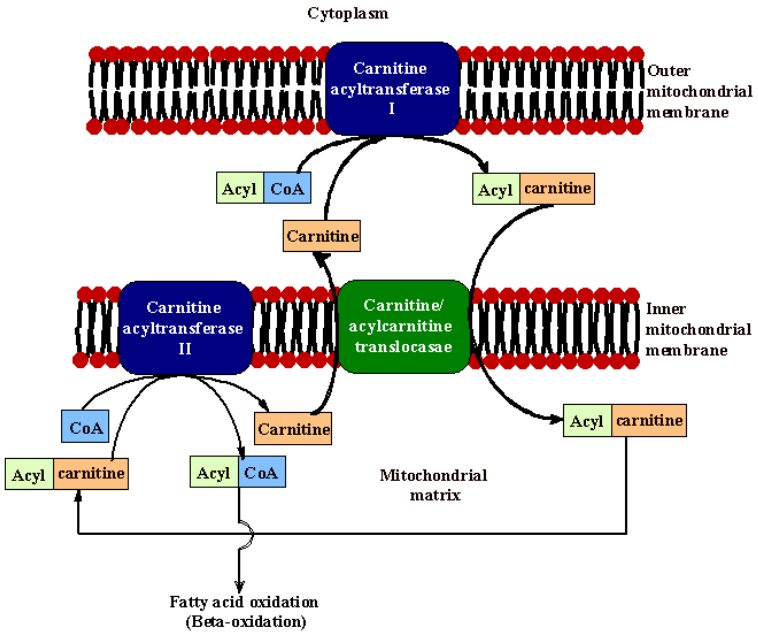

Figure 2.5 Transport of Acyl CoA into mitochondria during fatty acid oxidation (Anon, 2013c)

Subsequent to the transformation of acyl CoA to acylcarnitine, the transportation of acylcarnitin can occur across the inner mitochondrial membrane by a process called carnitine-acylcarnitine translocation in exchange for carnitine. Once inside the mitochondria, acylcarnitine are transformed back to acyl CoA by carnitine acyltransferase II, where after carnitine can be exchanged for another incoming molecule of acylcarnitine.

Step 3: Oxidation of Acyl CoA

The fatty acids (acyl CoA) which are now inside of the mitochondria are prepared to be oxidised by cleaving two carbons from the carboxyl end of acyl CoA (Fig 2.6). The broken bond which is between the alpha and beta carbons is referred to as beta oxidation. The beta oxidation products include the following:

- Acetyl CoA (which enters the citric acid cycle (Krebs cycle) for complete oxidation).
- NADH and $FADH_2$ (used for ATP production by means of the electron transport chain).
- Acyl CoA reduced by two carbons (ready for beta oxidation reaction system again).

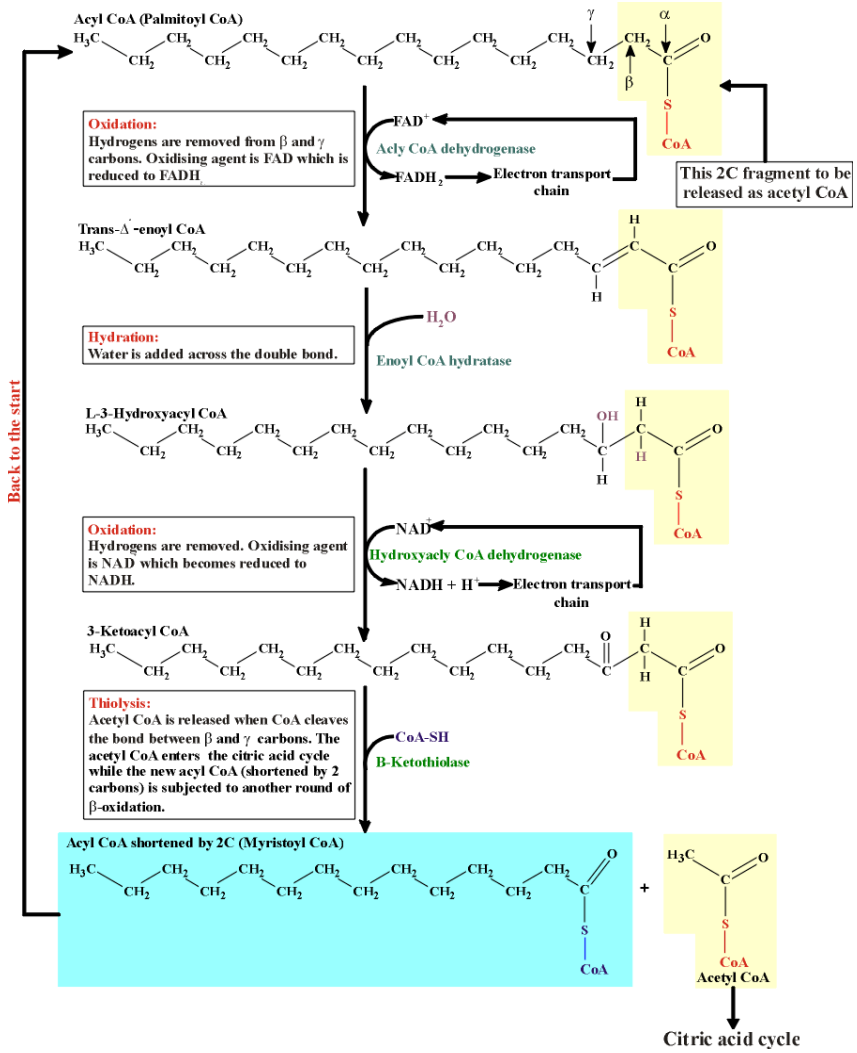

Fig 2.6 Oxidation of Acyl CoA (Anon, 2013c)

2.5 Lipid Peroxidation

Most microorganisms, animals and plants depend on oxygen to produce energy; however, high atmospheric oxygen concentration is potentially lethal (Knight, 1998). Valuable free radical research has been conducted in the last number of years and it enhances our understanding of the roles of free radicals in cell signalling as well as other physiological processes. It has been suggested that the effects of free radicals on muscle tissue depends on the production level and effectiveness of the defence mechanism of antioxidants.

Free radicals are either molecules, atoms or any other compounds having one or more unpaired electrons and are therefore extremely reactive and unstable, which makes them proficient of damaging biologically related molecules like DNA, lipids, carbohydrates and proteins. Free radicals, which attack the animal body, are often formed as an end product of metabolic activity.

The initial phase of lipid peroxidation involves the production of carbon-centred free radicals from a precursor molecule, for example a polyunsaturated fatty acid (PUFA):

$$LH \xrightarrow{\text{Initiator}} L^*$$

The next step is called the propagation phase and begins with the reaction of free radicals (L*) with oxygen and producing peroxyl:

$$L^* + O_2 \longrightarrow LOO^*$$

Once the propagation reaction has occurred, a somewhat unreactive carbon-centred radical (L*) is transformed to an extremely reactive peroxyl radical. The resulting peroxyl radical can attack any accessible peroxidizable material capable of peroxidation, subsequently creating hydroperoxide (LOOH) and a new carbon-centred radical (L*):

$$LOO^* + LH \longrightarrow LOOH + L^*$$

Lipid peroxidation is therefore a chain reaction and many cycles of peroxidation results in considerable damage to cells. The PUFA in membranes represents the peroxidizable material which is capable of peroxidation. It is generally acknowledged that the susceptibility to peroxidation of PUFA is proportional to the amount of double bonds in the molecules. Therefore docosahexaenoic fatty acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4-6) are amongst the most susceptible substrates for peroxidation in the membranes. It is important to highlight that the same PUFAs (DHA, 22:6n-3 and AA, 20:4-6) are also responsible for maintaining membrane properties including permeability and fluidity. Therefore, the functions and structure of PUFA are compromised as a result of lipid peroxidation within the biological membrane.

2.6 Antioxidants

Living organisms have developed specific antioxidant protective mechanisms to deal with ROS (Halliwell and Gutteridge, 1999). The term “antioxidant system” is used to describe these mechanisms. It is varied and accountable for the defence of cells from the actions of free radicals. This system includes:

- Water soluble antioxidants (uric acid, ascorbic acid, etc.)
- Natural fat soluble antioxidants (carotenoids, ubiquinones, vitamins A and E, ect.)
- Antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px)

To maximize the cellular protection, the protective antioxidant compounds are located in extracellular spaces, organelles or the subcellular compartments; therefore three major levels of defence are included in the antioxidant system of the living cell (Niki, 1996; Surai, 1999):

The inhibition of free radical formation is the first level of defence and is accomplished by deactivating catalysts or the removal of precursors of free radicals and consists of three antioxidants enzymes, namely

GSH-Px, CAT, SOD as well as metal binding proteins. The first level of antioxidant defence is unfortunately not sufficient to prevent free radical development completely, therefore lipid peroxidation and some peroxyl radicals do escape through the first defensive level of antioxidant safety screen.

As a result a second level of defence is required and consists of chain-breaking antioxidants, namely ascorbic acid, carotenoids, vitamin A and E, ubiquinol, uric acid, butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ) and butylated hydroxyanisol (BHA) (Kochhar and Rossell, 1990). Chain breaking antioxidants prevent peroxidation by keeping the propagation reaction chain length as short as possible. According to Gordon (1990), a substance will only be able to act as a chain-breaking antioxidant if a hydrogen atom can quickly be provided to a lipid radical and if the radical resulting from the antioxidant is steadier than the lipid radical, or is transformed to other stable products. Vitamin E is identified to be the most effective natural free radical scavenger to date and is considered the foremost chain breaking antioxidant in the cell. Nevertheless, the second level of antioxidant protection in the cell is incapable of stopping lipid peroxidation and several biological molecules do get damaged.

Therefore, a third level of defence is needed and is based on systems that remove the damaged molecules or repair them. The third level of antioxidant protection includes proteolytic (peptidase or proteases), lipolytic (lipases) and other enzymes, namely nucleases, proteinases, DNA repair enzymes, ligases, phospholipase, polymerases and numerous transferases.

2.6.1 Synthetic Antioxidants

Since the 1950's antioxidants have been increasingly used in the food industry to prolong the shelf life of food. Although many foodstuffs naturally contain antioxidants an artificial increase can further enhance the food quality by slowing down the oxidation process. Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tertiary butylhydroquinone (TBHQ), propyl gallate (PG) and ethoxyquin are common synthetic antioxidants used in the food production/processing industry for extending the shelf life of foods.

Anxiety about the safety of food additives has risen during the last few decades and renewed research on synthetic antioxidants has been conducted as a result of the increase in consumer resistance to additives. According to Botterweck *et al*. (2000), the epidemiological studies that were done on these antioxidants showed that these compounds cause no risk to human health. The US Food and Drug Administration have classified BHA, BHT and THBQ as "generally regarded as safe" (GRAS), at normal concentrations, while the US department of health's national toxicology program has recognized BHA as a substance "reasonably anticipated to be a human carcinogen").

Due to increased consumer awareness on the positive and negative effects of synthetic antioxidants extensive research has been conducted on the use of natural antioxidants in both food and other purposes. The value and benefits of natural ingredients as a food preservatives, was reported in both popular and scientific papers. According to Pratt and Hudson (1990), it is understandable to conclude that naturally occurring compounds in foods that have been eaten for thousands of years, will be safer than synthetic products.

2.6.2 Natural Antioxidants

In general, natural antioxidants have been found to have far few adverse effects compared to their synthetic equivalent. One such natural antioxidant L-Ascorbic acid, better known as Vitamin C acts as a catalyst for promoting the efficiency of other antioxidants (Specchio, 1992) and is found in a wide range of citrus fruit. Bermond (1990) noted that ascorbic acid (vitamin C) has been shown to delay age-related cataract development and prevent the carcinogenic and mutagenic action of N-nitroso compounds in the stomach. In addition to Bermond (1990), Block (1992) found that ascorbic acid also shows the ability to block different chemical carcinogens such as anthracene, 3, 4-benzpyrene, DDT and dieldrin.

Vitamin C is water soluble while another popular antioxidant, vitamin E is soluble in fat and therefore target different tissues (Best, 1992). Other natural antioxidants such as ß-carotene are capable of reducing singlet oxygen molecules and free radicals (Burton & Ingold, 1984), while several herbs and spices such as oregano, sage, rosemary, vanilla and tea extracts also possess antioxidant properties (Lindberg Madsen & Bertelsen, 1995; Shahidi, 2000). According to Specchio (1992), even onion peels have shown to have antioxidant actions.

The natural antioxidants that were tested in the present study were Vitamin E (mixed tocopherols), unfermented rooibos and chromium and are described in more detail below.

2.6.2.1 Vitamin E (tocopherols)

Vitamin E is found in plants and can be used as an antioxidant in food products that contain saturated animal fats. However, due to the unstable nature of vitamin E, it is often lost during processing (Specchio, 1992). As mentioned earlier, reactive oxygen species (ROS) are made continuously by the organism's normal oxygen metabolism. Fish tissue and plasma lipids are mainly prone to lipid peroxidation, due to their high amount of polyunsaturated fatty acids (PUFAs). Vitamin E has the ability to prevent lipid oxidation, thereby protecting bio membranes against oxidative damage (Waagbø *et al*., 1993; Lygren *et al*., 2000). Besides this function, vitamin E at high supplementation levels has been documented to improve immune responses of fish (Kiron *et al*., 2004).

2.6.2.2 Rooibos

Rooibos (*Aspalathus linearis)* leaves and their fine stems are used to produce unfermented and fermented rooibos tea (Bramati *et al*., 2003; Schulz *et al*., 2003; Juráni *et al*., 2008). The antioxidant activity of rooibos has been extensively investigated (Von Gadow *et al.,* 1996) and has a high antioxidant activity and is comparable to α-tocopherols, BHA and BHT (Von Gadow *et al*., 1997).

Flavonoids are plant pigments with antioxidant properties and are widely dispersed in nature, primarily represented by dihydrochalcones, flavones and flavonols however, aspalathin is exclusive to rooibos. A number of studies have demonstrated positive effects of several rooibos flavonoids and extracts (von Gadow *et al*., 1997; Juráni *et al*., 2008) which may be used for human and animal consumption.

2.6.2.3 Chromium

Chromium is a universal metal found at various concentrations in air, water, soil and essentially all biological tissues (Anderson, 1981). For the last two centuries, the existence of chromium has been known, however its use has been limited due to the belief that it is unsafe, even carcinogenic to living organisms. Recently, the importance of chromium for normal development and growth in humans and animals has been documented (Anderson, 1981; Gatta *et al*., 2000; Sahin *et al.,* 2002; Sahin *et al*., 2003Gatta *et al*., 2000). Animals identified as being chromium deficient have shown a reduced growth rate and life-span as well as a decreased tolerance to glucose (Gatta *et al*., 2000).

According to Sahin *et al*. (2003), the most important metabolic role of chromium is to potentiate insulin action through its presence in an organometallic molecule called glucose tolerance factor (GTF). It is well know that lipid peroxidation is influenced by insulin metabolism, therefore chromium (insulin cofactor), is assumed to limit the effects of diabetes.

2.7 Conclusion

Dietary antioxidants can protect against the development of oxidative stress and therefore decrease the development of several diseases (cancer, diabetes etc.) and enhance overall product quality and animal productivity (Hurley and Doane, 1989; McDowell, 2000). Meeting the optimal requirements for natural of farm animals is an important task for the animal nutritionist as correct management of nutrition can result in high reproductive and productive characteristics of farm animals.

Within South Africa, 60% of the abalone produced is destined for the live export market with the remaining being caned, dried or frozen. Throughout this process, the animals are extracted from their aquatic habitat and transported live for up to 40 hours. During this time, the abalone undergo extreme stress and can lose up to 15% of their body weight (moisture loss) (Vosloo & Vosloo, 2006). These losses in moisture are of major concern for the abalone farmer as they are paid on landed weight rather than harvest weight, which present a decrease in foreign revenue.

The aim of this study is therefore to determine if natural occurring antioxidants, namely unfermented rooibos, vitamin E (mixed tocopherols) and organic chromium, as well as different levels of polyunsaturated fatty acids can enhance the growth rate and reduce the moisture lost during live export, *ante mortem* and *post mortem* processing in the South African abalone, *Haliotis midae.*

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CHAPTER 3

THE GROWTH PERFORMANCE AND MEAT COMPOSITION OF THE SOUTH AFRICAN ABALONE, *HALIOTIS MIDAE* L., FED DIFFERENT FORMULATED DIETS

Abstract

Growth rate of South African abalone (*Haliotis midae*) has been extensively studied, however, only a few studies have evaluated the effect of low and high PUFA (Polyunsaturated Fatty Acids) levels and antioxidant additives. A six months feeding experiment was performed to determine the effects of fatty acid saturation and antioxidant supplementation on growth and meat quality of abalone. Experimental diets were maintained for six months and included a commercially available abalone diet (Control 1 – Marifeed's Abfeed® K26), a prototype abalone grower diet (Control 2 – NutroScience's AquaNutro Abalone grower diet), four high PUFA and four low PUFA diets (containing the NutroScience's AquaNutro as the base diet). Three treatment within the high and low PUFA diets were fortified with antioxidants (unfermented green rooibos, mixed tocopherols, chromium) whilst the fourth treatment had no antioxidants added. Abalone administered low PUFA diets had significantly higher growth, lower FCR and higher moisture retention compared to animals fed the high PUFA diet. The addition of antioxidants yielded mixed results however; there was some evidence that the integration of antioxidants improved moisture retention which may have consequences for meat quality. Interestingly, the commercially available feed (Control 1) yielded the greatest growth (W_F and % bodyweight gain) and FCR (Feed Conversion Rate) ($P<0.05$), the lowest % moisture and % protein content ($P>0.05$) and the highest % ash content ($P<0.05$) compared to the other treatments. Although not significant, Control 2 had the second best diet response (W_F, % weight gain, FCR, SGR_W and final condition factor), indicating potential for further research and development. The current study highlights the importance of understanding abalone nutrient requirements for developing an optimal diet for maximum production and quality.

Key words: abalone; *Haliotis midae*; growth; feed manipulation; PUFA; antioxidants

3.1 Introduction

Six indigenous abalone species are present within South African coastal zones and are distributed in both temperate water (12-21°C) from Port Nolloth on the west coast (Atlantic ocean) to tropical water up to East London on the east coast (Indian ocean) (Elliott 2000; Franchini *et al*., 2011; van Schalwyk, 2011). Due to its fast growth rate, large size at age and acceptable market characteristics (colour and taste) *H. midae* L. has been exploited as a fisheries resource and is also of commercial significance within the aquaculture industry (Hecht, 1994; Sales & Britz, 2001; Naidoo *et al*., 2006; Franchini *et al*., 2011; van Schalkwyk, 2011). As a result of its status as a delicacy in the East Asian markets (Naidoo *et al*., 2006; ten Doeschate & Coyne, 2008), *H. midae* can reach premium prices of between $35 and $45 per kg live weight (Raemaekers *et al*., 2011).

Aquaculture of *H. midae*, has increased significantly since the closure of wild fisheries in 2008 (Raemaekers *et al*., 2011) and currently represents 94% of the total marine aquaculture in South Africa (DAFF, 2012), resulting in significant generation of foreign income (Marcey & Coyne, 2005; ten Doeschate & Coyne, 2008). Subsequent to the decline in the wild fisheries resources, the culture of abalone in South Africa has developed into the third largest producer of abalone in the world with 14 farms producing 1036 tonnes in 2011 (DAFF, 2012). In order to enable continuous growth within the industry improvements in technology and the enhancement of knowledge is necessary throughout the abalone production process, from seeding to final point delivery. One aspect that has gained increasing attention is the use of diet manipulation which can be used to enhance the growth and quality (sensory perception, composition etc) of abalone (Bautista-Teruel *et al*., 2003; Gomez-Montes *et al*., 2003; Naidoo *et al*., 2006; ten Doeschate & Coyne, 2008).

The South African abalone, *H. midae* can reach a maximum shell length of about 200mm in the wild, at an estimated age of up to 30 years (Sales and Britz, 2001; Naidoo *et al*., 2006), however individuals grown within an aquaculture facility exhibit enhanced growth rates, reaching marketable size of (80-100mm) in a period of four to five years (Sales and Britz, 2001; Naidoo *et al*., 2006; ten Doeschate & Coyne, 2008). Although abalone have a significantly higher growth rate under commercial conditions, growth rates can still vary depending on a number of factors such as water quality and temperature, stocking density and diet (Stuart & Brown, 1994; Guzmán & Viena, 1998; Naidoo *et al*., 2006). Such environmental effects can hamper production and poses further challenges to the farmer (Naidoo *et al*., 2006). In order to remain competitive and meet the high international market demand, it is important to produce marketable abalone of good quality in the shortest time possible. Such practice leads to lower production cost and an increase in turnover (Naidoo *et al*., 2006; ten Doeschate & Coyne, 2008).

The rate of abalone growth can be significantly altered by diet and feeding strategies (Leighton, 1974; Britz, 1996a & 1996b; Fleming *et al*., 1996; Guzmán & Viana, 1998; Shpigel *et al*., 1999; Boarder & Shpigel, 2001; Bautista-Teruel *et al*., 2003; Gomez-Montes *et al*., 2003; Naidoo *et al*., 2006; ten Doeschate & Coyne, 2008) therefore optimisation of the nutritional diet can promote growth and overall harvest. Although all dietary components such as protein, vitamins, minerals and carbohydrates are important for normal cell functioning, lipids are considered the most important energy source in animal muscle. Nonetheless, fatty acid composition has remained largely unstudied in molluscs (Durazo-Beltrán *et al*., 2003) and only few studies have examined the essential fatty acids linoleic (n-6) and linolenic (n-3) which are important for growth and reproduction (Caers *et al*., 2000; Navarro and Villanueva, 2000; Nelson *et al*., 2002; Durazo-Beltrán *et al*., 2003). Although herbivores can synthesize lipids *de novo*, some lipids are essential (linoleic acid, linolenic acid and arachidonic acid) and can only be derived directly from the diet (Nelson *et al*., 2002).

Despite the importance of lipids in abalone diet, a high fat diet can intensify oxidative stress, which can negatively influences the integrity of the membranes, subsequently reducing the quality of the abalone meat (Blokhina *et al.,* 2002; Catalá, 2009). However, antioxidants have been shown to prevent peroxidation, which in turn minimises cell membrane oxidation and reduces cell membrane permeability (better water holding capacity) (Cannon *et al.*, 1995; Cannon *et al.*, 1996; Ibrahim e*t al.,* 1997; Jensen *et al.*, 1998;

Jenkinson *et al.*, 1999; Eritsland, 2000; Blokhina *et al.,* 2002; Catalá, 2009). The addition of antioxidants into abalone formulated diet may therefore be advantageous but requires further investigation.

Due to the high price associated with using commercially formulated feed, a number of farmers have developed in house diets in order to lower cost. However, an in-depth understanding of the nutritional requirements and physiological processes of abalone is important in order to fulfil the nutritional requirements and optimise feeding strategies (Smith, 1989; Gomez-Montes *et al*., 2003). Although extensive research has been conducted on abalone growth few studies have looked at the effects of polyunsaturated fatty acids and antioxidant additives on growth and quality of adult *H. midae* Therefore, the aim of this study was to investigate how polyunsaturated fatty acid levels and antioxidant additives can affect a) growth and b) meat composition of the native South African abalone *H. midae.*

3.2 Materials & Methods

3.2.1 Experimental Unit and Animals

The trial was conducted at Irvin & Johnson's Abalone Culture Division at Danger Point, Gansbaai, South Africa using a combination of a flow through (30%) and recirculatory (70%) system - contained within 3 concrete tanks (Fig. 3.1). The concrete tanks were designed to have one inflow and one outflow channel located at opposite ends of the tanks which can lead to differences in water quality within a tank system, subsequently affecting abalone growth depending on the location of the basket. Individuals within the same age group with similar mean shell length (64.96mm; S.E.M 0.237) and body weight (57.38g; S.E.M 0.293) were used for the experiment to eliminate the possibility of size bias. The experiment consisted of 10 feed treatments with five replicates within each treatment. Each replicate contained 225 individual abalone which represented normal stocking densities on the farm.

Treatments were randomly allocated within each tank (Fig. 3.1) and once a week the positions of treatment/baskets were rotated to eliminate the effect of water quality on growth. The experiment was conducted over a six month period during which time the animals were managed according to normal commercial farm conditions.

Figure 3.1 The experimental system consisting of concrete tanks containing the abalone baskets (replicates).

3.2.2 Treatments

The ten feed treatments were divided into 3 main feeding categories: four treatments were fed high PUFA diets, four treatments contained feeds with low PUFA diets while two treatments were used as controls (Table. 3.1). The controls consisted of two commercially used feeds, namely:

- Abfeed® K26 (Stil Street, Lot 2A, New Harbour, Hermanus, Western Cape, South Africa) is the most common artificial food used in South Africa and as standard feeding practice at the experimental site. The feed contains 25-26% protein, 1.2% fat and 0.9% fiber (Naidoo *et al*., 2006).
- AquaNutro (5 Nywerheid Crescent, Malmesbury Western Cape, South Africa) feed contains 35% protein, 5% fat and 3% fibre and was used as a base diet for all experimental feeds.

AquaNutro was used as the base diet for all treatments within the high and low PUFA groups. Sunflower oil (PUFA 65%; Anon, 2013a) was added to the base diet of four treatments to create a high PUFA feed whilst beef tallow (PUFA 4%; Anon, 2013b) was added to the remaining four treatments to create a low PUFA diets.

Three of the high and low PUFA treatments had additives with antioxidant properties integrated into their diet and contained DSM's vitamin E in the form of mixed tocopherols with 95% activity (M), Afriplex's 90% unfermented rooibos extract (R) and Alltech's Bio-Chrome with 0.2% activity (Cr), while the fourth treatment in each PUFA group had no additives added (HN and LN).

The ten treatments/diets will be referred to as Control 1 for Abfeed® K26, Control 2 for AquaNutro, LM, LR and LCr for the low PUFA with additives diets, LN for low PUFA with no additive diet, HM, HR and HCr for the high PUFA with additives diets and HN for high PUFA with no additive diet. The experimental treatments are outlined in Table 3.1.

Table 3.1 The dietary treatments fed to the experimental animals for the 6 month feeding trial.

Diet group and description	Treatment Identification	Supplement Concentration (g/kg diet)
Abfeed®	Control 1	-
AquaNutro	Control 2	-
High PUFA diets		
No Additives	HN	-
Mixed Tocopherols	HM	0.0376
Green Rooibos	HR	0.05
Chromium	HCr	0.2
Low PUFA diets		
No Additives	LN	-
Mixed Tocopherols	LM	0.0376
Green Rooibos	LR	0.05
Chromium	LCr	0.2

3.2.3 Measuring Abalone Growth Performance

On day one of the experiment and every 30 days thereafter 25 individuals were randomly sampled from each replicate to measure growth performance. The length of the shell was measured to the nearest 1 mm and the weight was taken to the nearest 0.1 g. After the measurements were taken, each abalone was marked with putty on the shell to avoid measurement duplication. This practice eliminated the factor of handling stress on the growth performance of the abalone as all animals were handled just once. Weight was measure over the entire six month period but due to technical issues (callipers were not correctly calibrated and gave incorrect measurements) length was only measured for the first five months.

3.2.3.1 Body Weight and Shell Length Gain

The efficiency of the diet in relation to growth performance was evaluated and determined as the percentage body weight and shell length gain relative to the initial values (day 0) and was calculated as follows:

Percentage Body Weight Gain = $((W_F - W_I) / W_I) \times 100$
Percentage Shell Length Gain = $((L_F - L_I) / L_I) \times 100$

Where;
W_I = mean initial body weight (g)

W_F = mean final body weight (g)
L_I = mean initial shell length (mm)
L_F = mean final shell length (mm)

3.2.3.3 Specific Growth Rate

Exponential transformation was used to determine specific growth rates. For specific growth rate (SGR, % body weight and length gain per day), the logarithm of weight / length gained was divided by the duration of the culture period in days and converted to percentage. The equation is as follow:

Body Weight:

$SGR = [\{ \ln(W_F) - \ln(W_I) \} / t] \times 100$

Where;

$\ln(W_I)$ = the log of the mean initial body weight (g)
$\ln(W_F)$ = the log of the mean final body weight (g)
t = the time in days

Shell Length:

$SGR = [\{ \ln(L_F) - \ln(L_I) \} / t] \times 100$

Where;

$\ln(L_I)$ = the log of the mean initial shell length (mm)
$\ln(L_F)$ = the log of the mean final shell length (mm)
t = the time in days

3.2.3.4 Feed Conversion Ratio

In animal husbandry, the feed conversion ratio (FCR) is a measure of an animal's efficiency in converting feed mass into increased body mass. The optimum FCR is ≤1, where 1 gram of feed is converted into ≤1gram of body weight. It was calculated as follows:

FCR = dry feed weight fed (g) / abalone wet weight gain (g)

3.2.3.5 Condition Factor

The condition factor is a concept developed to determine the health of individual abalone by examining the weight of the abalone per unit shell length (Britz, 1996b). The condition factor was calculated at the beginning (n=25 per replicate) and at completion (n=25 per replicate) of the experiment. The condition factor was calculated as follow (Britz, 1996b):

Condition Factor = (body weight (g) / shell length (mm))$^{2.99}$ x 5575

3.2.4 Abalone proximate analysis

A total of 80 animals were sampled at the end of the trial for proximate analysis; 8 individuals were randomly selected from each treatment. They were immediately shucked (separate the shell from the body), intestines removed and washed. The animals from the different treatments were stored in separate plastic bags on ice and transported to the laboratory. Once in the laboratory the animals were frozen at -20°C until proximate analyses were performed. The samples were defrosted prior to homogenization and analysis of the sample.

A 2.5g sample of homogenized tissue was used to determine ash and moisture content using the methodology as outlined by AOAC International (2002a, b) Official Method 942.05 and Official Method 934.01, respectively. The sample was placed in a porcelain crucible and positioned in a drying oven at 105°C for 24h. Subsequent to the drying period moisture content was calculated by subtracting the dried weight from the initial weight (2.5g). Ash determination was achieved similarly, by placing the dried sample in an oven at 500°C for 6h and subtracting final weight from initial weight (2.5g).

Crude fat was determined by the methodology outlined by Lee *et al.,* 1996. A mixture of 5g homogenized abalone tissue and 50ml chloroform:methanol (1:2, v/v) was mixed with a Bamix in a glass beaker for 1 minute. The mixture was then filtered through a Whatman No. 1 filter paper into separator funnels. After filtration, 20ml 0.5% NaCl was added and the solution was mixed. After 30 minutes, the moisture and fat separated and 5ml of the bottom section were used to determine the crude fat content.

The meat residue which remained on the filter paper subsequent to filtration in the fat analysis was used to measure crude protein and amino acid content. The meat residue was dried and ground to a powder. 0.1g of the dried residue was used to determine protein content according to the methodology as outlined by AOAC International (1992) Official Method 992.15 by using a FP-528 protein/nitrogen determinator (Leco Corporation, St Joseph, MI, USA). Both the crude fat and protein measurements were duplicated and the means were calculated for further analysis.

3.2.5 Fatty and amino acid analysis

Due to cost constraints a limited number of treatments and abalone could be sampled for fatty and amino acid analysis. A parallel study conducted by the author examined the rate and extent of moisture loss of abalone under identical experimental conditions (Chapter 4). From that study, four treatments (Control 2, LN, LM and HM) were identified as having superior moisture retention. Five animals were randomly selected from Control 1 (Abfeed®) and each of the high moisture retention treatments (Control 2, LN, LM and HM) and prepared for fatty and amino acid analysis. All fatty and amino acid lab analysis was conducted only once and therefore did not contain replicates of analysis.

3.2.5.1 Fatty acid analysis

To extract fatty acids, bonds need to be broken between the lipids. The fatty acids are methylated and then analyzed by gas chromatography according to the methodology outlined by Folch *et al*. (1957), Heimann (1980) and Pomeranz and Meloan (1971).

A subsample of the homogenate (2g) was used to determine the percentage of fatty acid using the extraction protocol as described by Folch *et al*. (1957). The sample of homogenate was added to an extraction tube and the extraction solvent was added, 20ml chloroform:methanol (C:M 2:1; v/v). All extraction solvents contained 0.01% butylated hydroxytoluene (BHT) as an antioxidant. Heptadecanoic acid (C17:0; 500µL 10mg/ml) was used as an internal standard to quantify the individual fatty acids. A polytron mixer was used to homogenise the sample within the extraction solvent. The solution was transferred through an extraction funnel into a 50ml volumetric flask, using a glass microfibre filter. The extraction tube was rinsed with 20ml C:M (2:1; v/v) and the solution transferred through the extraction funnel. The extraction funnels were placed under a vacuum to dry the remaining filtrate. The volumetric flask was filled to 50ml with C:M (2:1; v/v), mixed and 100µL was transferred to a spotting tube and dried under nitrogen. The lipids isolated were transmethylated adding 2ml transmethylating reagent (methanol:sulphuric acid; 19:1; v/v) for 2h at 70°C. After cooling, the resulting fatty acid methyl esters (FAME) were extracted with 1ml H_2O and 2ml hexane. The top hexane phase was transferred to a spotting tube and dried under nitrogen.

After being dried under nitrogen, 50µL Hexane was added and 1µL analysed by gas liquid chromatography (GC) (Thermo Focus GC equipped with flame ionisation detection) using a BPX70, 60 mm x 0.25mm internal diameter, 0.25µm film thickness capillary column (SGE, Australia). Gas flow rates were: hydrogen, 25ml/min and hydrogen carrier gas, 1ml/min. Temperature programming was linear at 7°C/min: initial temperature 60°C; final temperature 160°C; injector temperature 220°C and detector temperature 260°C. The FAME were identified by comparison of the retention times to those of a standard 37 Component FAME mixture (10 mg/ml in CH_2Cl_2, Supelco product number 47885-U) and the milligram fatty acid/g of tissue sample was calculated.

3.2.5.2 Amino acid analysis

Amino acid content was determined by hydrolyzing a sample of protein according to the methodology outlined by AOAC (2003) Official method 994.12. Samples were weighed (0.1g) and hydrolyzed for 24h at 110°C in 6ml of 6 N HCl and 15% phenol. Hydrolyzed samples were stored at -20°C. From this, the amino acids were isolated by means of HPLC under gradient conditions and purified by partition chromatography (Cowey & Cho, 1993).

Before use, samples were thawed to room temperature. Each sample was mixed on a vortex for 5-10 seconds and centrifuged at 15000g for 5 minutes (Hermle bench centrifuge). The supernatant (25µL) was measured with a Hamilton syringe and placed into a glass hydrolysis tube. These were dried under vacuum for 1h, and derivatised. The dried solution (methanol:water:triethylamine 2:2:1) was added (20µL) to adjust pH and dried for 1h. The sample was derivatised by adding 20µL derivatising solution (methanol:water:triethylamine:phenylisothiocyanate 7:1:1:1) and allowed to react for 10min at room temperature, and dried under vacuum for a minimum of 1h and a maximum of 3h till dry. The dried sample was re-suspended in 400µl of Picotag sample diluent (Waters, Milford, MA, USA). A portion of the sample (16µL) was separated by HPLC under gradient conditions, where Buffer A was a sodium acetate buffer (pH

6.4) containing 5000 ppm EDTA, 1:2000 triethylamine and 6% acetonitrile and Buffer B a 60% acetonitrile buffer containing 5000 ppm EDTA. The obtained data was analysed using Breeze software (Waters, USA).

3.3 Statistical analysis

All data analyses were performed using SAS for Windows version 9.1.3 (SAS Institute Inc., USA) and STATISTICA for Windows version 11 (StatSoft. Inc., USA). Interactions between additives within the high and low PUFA levels were examined. Where no interactions existed a GLM was performed to determine if differences existed between PUFA levels and between treatments. Due to the small number of treatments (1 high PUFA and 2 low PUFA treatments) and animals (n=5) within each treatment, fatty acid and amino acid analysis (ANOVA) was conducted between treatments and not between PUFA levels.

3.4 Results

No significant interaction ($P>0.05$) between treatments within the PUFA levels for W_I, W_F, L_I and % weight gain was found, however this was not the case for L_F and % length gain where a significant interaction ($P<0.05$) was observed. Therefore analysis at the PUFA level for L_F and % length gain could not be carried out. Analysis of variation between the high and low PUFA levels revealed no significant differences ($P>0.05$) between PUFA levels for W_I, L_I and % weight gain; however, low PUFA animals had significantly higher final weight (Table 3.2a).

Table 3.2a Summary of the average initial and final wet weights (W_I, W_F), initial length (L_I), and mean % weight gain of *H. midae* fed two different levels of PUFA diets. All values are given as mean ± standard error of the mean (S.E.M.)

Treatments	Avg W_I	Avg W_F	Avg L_I	% Weight Gain
High PUFA	57.25 ± 0.065	81.68^{a} ± 0.879	64.80 ± 0.492	43.15 ± 1.574
Low PUFA	57.53 ± 0.063	84.28^{b} ± 0.589	64.88 ± 0.499	47.05 ± 1.032

^{ab}Means with the different letters in a column denote significant differences ($P<0.05$)

A number of differences were observed between treatments for final weight (W_F), % weight gain and initial length (L_I), however few patterns were detected. At the end of the 6 month feeding trial, the abalone from Control 1 had a significantly higher final weight and % weight gain ($P<0.05$) compared to all other treatments (Table 3.2b). Although not significant, HCr was found to have the lowest final weight and length. No significant differences between treatments were found for initial weight, final length and % length gain.

Table 3.2b Average initial and final wet weights (W_I, W_F) and lengths (L_I, L_F), mean % weight and length gain of *H. midae* fed 10 different formulated diets. All values are given as mean ± standard error of the mean (S.E.M.)

Treatment	Avg W_I	Avg W_F*	% Weight Gain*	Avg L_I	Avg L_F**	% Length Gain**
Control 1	57.2 ± 0.297	95.5^{a} ± 1.126	67.8^{a} ± 2.219	65.4^{a} ± 0.239	76.8 ± 1.021	17.5 ± 1.734
Control 2	57.7 ± 0.294	88.6^{b} ± 1.004	54.4^{b} ± 2.056	65.4^{a} ± 0.228	74.7 ± 0.484	14.4 ± 0.548
HN	57.2 ± 0.289	83.9^{cd} ± 1.004	47.1^{bcd} ± 1.842	63.9^{c} ± 0.258	74.6 ± 0.690	16.9 ± 2.148
HM	57.1 ± 0.276	81.6^{cd} ± 0.870	43.2^{cd} ± 1.647	65.2^{ab} ± 0.214	74.1 ± 0.485	12.9 ± 1.493
HR	57.3 ± 0.313	81.6^{cd} ± 0.831	42.9^{cd} ± 1.711	64.1^{bc} ± 0.228	73.6 ± 0.491	14.9 ± 0.905
HCr	57.4 ± 0.301	79.6^{d} ± 0.878	39.4^{d} ± 1.764	66.0^{a} ± 0.220	73.5 ± 0.539	11.6 ± 1.215
LN	57.4 ± 0.283	85.4^{bc} ± 0.762	49.2^{bc} ± 1.450	65.2^{ab} ± 0.242	75.0 ± 0.551	15.2 ± 1.419
LM	57.5 ± 0.290	84.1^{c} ± 0.803	46.9^{bcd} ± 1.559	65.6^{a} ± 0.233	75.0 ± 0.785	14.5 ± 1.923
LR	57.5 ± 0.258	84.9^{bc} ± 0.908	47.8^{bc} ± 1.612	65.3^{a} ± 0.273	74.5 ± 0.626	14.5 ± 1.965
LCr	57.7 ± 0.330	82.7^{cd} ± 0.902	44.3^{cd} ± 1.875	63.4^{c} ± 0.238	74.7 ± 0.588	18.1 ± 1.906
P-Value	0.8777	<.0001	<.0001	<.0001	0.07	0.15

$^{a-f}$Means with the different letters in a column differ significantly (P<0.05)
*Values calculated over a 185 day period
**Values calculated over a 158 day period

No interactions were found between treatments within each PUFA group for FCR, SGR_W, SGR_L or the condition factor. Abalone administered the low PUFA diet had a significantly lower FCR ($P<0.05$) while no differences between PUFA groups ($P>0.05$) was found for the other variables (SGR_W, SGR_L and condition factor) (Table 3.3a).

Table 3.3a Feed conversion ratio (FCR), average specific growth rate for body weight (SGR_W), shell length (SGR_L) and condition factor for *H. midae* fed two different levels of PUFA diets. All values in the table are represented as means ± standard error of the mean (S.E.M.)

Treatments	FCR	SGR_W	SGR_L	Condition Factor Day 1	Condition Factor Day 158
High PUFA	2.19^{a} ± 0.038	0.192 ± 0.0060	0.083 ± 0.0061	1.23 ± 0.028	1.12 ± 0.0087
Low PUFA	1.99^{b} ± 0.015	0.207 ± 0.0043	0.090 ± 0.0047	1.24 ± 0.029	1.13 ± 0.0080

^{ab}Means with the different letters in a column differ significantly ($P<0.05$)

As shown in Table 3.3b abalone fed the Control 1 diet had a significantly lower FCR compared to all other treatments ($P<0.05$) and also had the highest SGR_W, differing significantly from HM, HR, HCr and LCr ($P<0.05$). In addition, HCr had significantly higher FCR compared to treatments LN and Control 2 ($P<0.05$). No differences were found in SGR_L between treatments ($P>0.05$). Numerous differences were observed between diets for both condition factors (CF_I and CF_F) and although no results stand out it was observed that both controls had the highest CF_F.

Table 3.3b Feed conversion ratio (FCR), average specific growth rate for weight (SGR_W), shell length (SGR_L) and condition for *H. midae* fed 10 different formulated diets. All values in the table are presented as means ± standard error of the mean (S.E.M.)

Treatment	FCR	SGR_W	SGR_L	Condition Factor Day 1	Condition Factor Day 158
Control 1	1.47^{c} ± 0.062	0.277^{a} ± 0.0179	0.101 ± 0.0093	1.205^{c} ± 0.0116	1.201^{a} ± 0.0302
Control 2	1.79^{b} ± 0.064	0.231^{ab} ± 0.0239	0.084 ± 0.0029	1.206^{c} ± 0.0130	1.169^{ab} ± 0.0082
HN	2.08^{a} ± 0.087	0.206^{ab} ± 0.0123	0.097 ± 0.0117	1.283^{a} ± 0.0146	1.095^{d} ± 0.0142
HM	2.21^{a} ± 0.065	0.193^{b} ± 0.0094	0.081 ± 0.0053	1.202^{c} ± 0.0107	1.115^{cd} ± 0.0115
HR	2.25^{a} ± 0.064	0.190^{b} ± 0.0134	0.087 ± 0.0050	1.272^{ab} ± 0.0133	1.133^{cdb} ± 0.0069
HCr	2.22^{a} ± 0.069	0.177^{b} ± 0.0146	0.068 ± 0.0070	1.168^{c} ± 0.0120	1.130^{cdb} ± 0.0124
LN	1.99^{ab} ± 0.062	0.215^{ab} ± 0.0119	0.089 ± 0.0076	1.215^{bc} ± 0.0132	1.149^{cb} ± 0.0153
LM	1.97^{ab} ± 0.064	0.206^{ab} ± 0.0115	0.084 ± 0.0106	1.192^{c} ± 0.0119	1.112^{cd} ± 0.0256
LR	1.96^{ab} ± 0.064	0.210^{ab} ± 0.0100	0.084 ± 0.0105	1.218^{bc} ± 0.0158	1.136^{cdb} ± 0.0161
LCr	2.03^{ab} ± 0.066	0.195^{b} ± 0.0133	0.104 ± 0.0101	1.324^{a} ± 0.0139	1.123^{cdb} ± 0.0132
P-Value	<.0001	0.0017	0.17	<.0001	<.01

$^{a-e}$Means with the different letters in a column differ significantly ($P<0.05$)

Overall a consistent increase in weight was observed during the experimental period (Fig. 3.2a). It is clear from Fig. 3.2a that Control 1 increased in weight shortly after the experiment begun and continued to display higher weight gain compared to the other treatments.

In general it was observed that most treatments (excluding Control 1) grew equally (Fig 3.2b). Subsequent to August, Control 1 on average had a greater increase in shell length when compared to all other treatments.

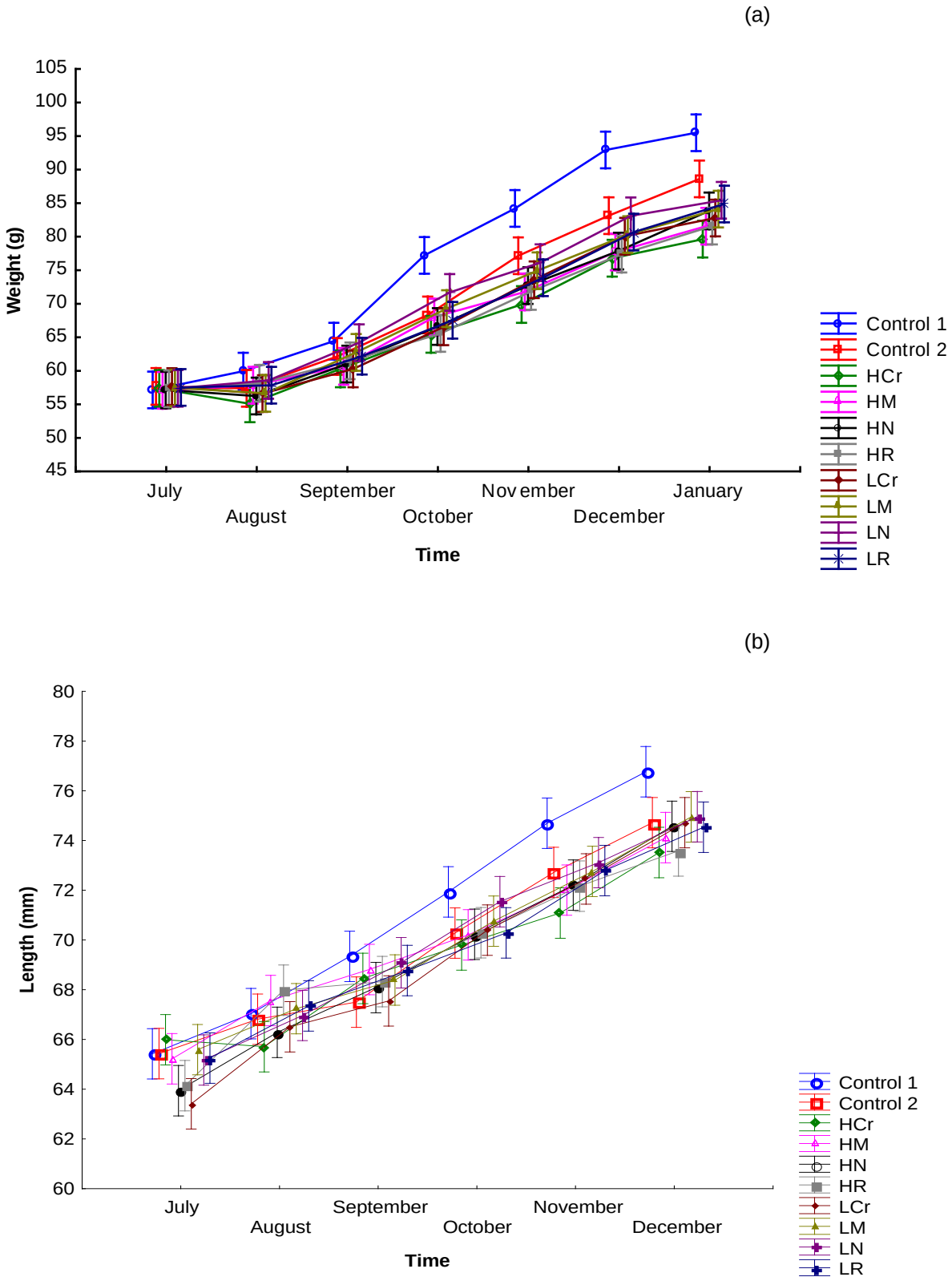

Figure 3.2 Line chart displaying temporal variability in weight (a) and length (b) of *H. midae* fed 10 different formulated diets. The error bars denote 0.95 confidence intervals.

No interactions within PUFA levels or significant differences between PUFA levels were found for % moisture, % ash and % protein, however a significant interaction within each PUFA level was observed for % fat.

The percentage of moisture and protein remaining subsequent to the experiment was lowest in Control 1 and differed significantly (P<0.05) from most treatments (excluding HN for % moisture and HN and LN for % protein) (Table 3.4). Control 1 also had a significantly higher percentage of ash content when compared to all other treatments (P<0.05). The low PUFA diet with added Rooibos had the highest moisture content of all treatments

Abalone fed the LM diet were found to have the highest percentage fat content (differing significantly from LR, HCr and Control 2 (P<0.05)), whereas animals fed the LR diet had the lowest percentage fat content and differed from most other diets (P<0.05) (excluding HCr).

Control 2 had the highest percentage of protein content, and differed significantly from treatments LR, LN, HN and Control 1 (P<0.05). A statistical summary including average percentage moisture, ash, fat and protein can be seen in Table 3.4 below.

Table 3.4 Proximate composition of *H. midae* fed 10 different formulated diets. All values in table are displayed as mean ± standard error of the mean (S.E.M.)

Treatments	% Moisture	% Protein	% Ash	% Fat
Control 1	69.80^{d} ± 0.327	18.79^{c} ± 0.257	2.60^{a} ± 0.074	1.33^{a} ± 0.056
Control 2	71.46^{bc} ± 0.305	21.25^{a} ± 0.231	2.11^{b} ± 0.060	1.00^{bc} ± 0.091
HN	70.80^{cd} ± 0.315	19.89^{bc} ± 0.239	1.94^{bc} ± 0.062	1.44^{a} ± 0.046
HM	72.49^{ab} ± 0.305	20.74^{ab} ± 0.231	2.15^{b} ± 0.058	1.24^{ab} ± 0.044
HR	72.35^{ab} ± 0.339	20.95^{ab} ± 0.257	1.97^{bc} ± 0.067	1.18^{ab} ± 0.099
HCr	72.41^{ab} ± 0.305	20.72^{ab} ± 0.231	1.82^{c} ± 0.058	0.75^{cd} ± 0.061
LN	71.56^{bc} ± 0.305	18.90^{c} ± 0.231	2.12^{b} ± 0.060	1.35^{a} ± 0.072
LM	71.63^{bc} ± 0.305	20.27^{ab} ± 0.247	1.81^{c} ± 0.058	1.50^{a} ± 0.152
LR	73.46^{a} ± 0.305	20.04^{b} ± 0.257	1.87^{bc} ± 0.058	0.40^{d} ± 0.113
LCr	71.81^{bc} ± 0.305	20.32^{ab} ± 0.231	2.08^{bc} ± 0.058	1.49^{a} ± 0.090
P-Value	<.0001	<.0001	<.0001	<.0001

$^{a-d}$Means with the different letters in a column differ significantly (P<0.05)

In Table 3.5, a summary of statistical results and mean percentage fatty acid composition are outlined for the five treatments analysed. As discussed earlier only 5 treatments (best performing treatments as outlined in Chapter 4) were analysis due to financial and logistical constraints. The only significant differences between treatments were obtained in the fatty acid 18:2 n-6, 20:2 and n6:n3. Although variation existed between treatments for fatty acid 18:2 n-6, it was found that Control 2 had the lowest percentage of 18:2 n-6 differing significantly from treatments HM and Control 1. Animals fed the Control 1 diet were found to have a significantly higher (P<0.05) percentage of 20:2 fatty acid when compared to the other diets.

Furthermore, there were differences (P<0.05) between treatments in the n6/n3 ratios, where Control 1 and HM differ significantly from treatments LM, LN and Control 2. Overall Control 1 contained the highest % SFA and lowest MUFA while Control 2 had the lowest % PUFA and PUFA: SFA, although these differences were not significant the overall results indicate the importance of feed type in abalone fatty acid composition.

Table 3.5 Fatty acid composition (percentage) of *H. midae* meat fed five different treatments. All values in the table are given as means ± standard error of the mean (S.E.M.)

Fatty Acids	Control 1	Control 2	LN	LM	HM	P-value
14:0	0.96 ± 0.216	1.16 ± 0.446	1.80 ± 0.459	1.06 ± 0.242	0.80 ± 0.247	0.311
15:0	1.62 ± 0.657	0.72 ± 0.120	0.78 ± 0.120	0.66 ± 0.081	0.78 ± 0.200	0.221
16:0	26.86 ± 4.536	24.62 ± 3.102	26.78 ± 4.168	24.48 ± 1.736	21.72 ± 2.495	0.813
16:1	0.74 ± 0.087	0.86 ± 0.098	1.12 ± 0.162	0.98 ± 0.097	0.82 ± 0.132	0.221
18:0	19.22 ± 2.753	15.64 ± 2.028	14.72 ± 1.555	16.10 ± 0.814	15.22 ± 1.448	0.465
18:1 n-9t	1.16 ± 0.317	1.16 ± 0.685	0.40 ± 0.055	0.56 ± 0.024	0.38 ± 0.049	0.281
18:1 n-9c	9.32 ± 1.036	21.04 ± 5.222	13.84 ± 1.465	13.62 ± 0.765	22.70 ± 6.343	0.106
18:2 n-6c	5.00^{a} ± 0.756	3.50^{b} ± 0.623	3.76^{ab} ± 0.304	4.44^{ab} ± 0.316	5.60^{a} ± 0.367	0.047
18:3 n-6	1.02 ± 0.174	0.60 ± 0.084	0.62 ± 0.107	0.72 ± 0.049	0.68 ± 0.169	0.165
18:3 n-3	1.76 ± 0.160	1.42 ± 0.156	1.48 ± 0.213	1.50 ± 0.155	1.74 ± 0.242	0.599
20:1	0.26 ± 0.040	0.30 ± 0.032	0.24 ± 0.024	0.32 ± 0.058	0.28 ± 0.037	0.650
20:2	1.84^{a} ± 0.363	0.92^{b} ± 0.146	0.94^{b} ± 0.172	0.94^{b} ± 0.040	1.16^{b} ± 0.229	0.033
20:3 n-6	7.62 ± 1.333	6.10 ± 0.807	7.04 ± 1.127	7.68 ± 0.893	6.84 ± 0.671	0.791
20:3 n-3	0.22 ± 0.049	0.72 ± 0.124	0.14 ± 0.024	0.24 ± 0.040	0.42 ± 0.102	0.066
20:4 n-6	1.54 ± 0.201	1.28 ± 0.150	1.34 ± 0.266	1.62 ± 0.080	1.12 ± 0.080	0.274
20:5 n-3	1.02 ± 0.196	1.04 ± 0.206	1.90 ± 1.030	1.12 ± 0.153	1.02 ± 0.037	0.649
21:0	0.26 ± 0.040	0.30 ± 0.032	0.24 ± 0.024	0.32 ± 0.058	0.28 ± 0.037	0.650
22:5 n-3	16.40 ± 2.896	16.66 ± 2.796	19.68 ± 3.027	20.52 ± 1.446	16.22 ± 1.746	0.613
22:6 n-3	3.38 ± 0.312	2.52 ± 0.407	3.42 ± 0.508	3.44 ± 0.650	2.52 ± 0.265	0.349
∑ SFA	48.96 ± 6.685	42.45 ± 5.377	44.35 ± 5.384	42.60 ± 2.593	38.78 ± 4.231	0.710
∑ MUFA	10.32 ± 1.053	22.21 ± 5.124	15.21 ± 1.615	14.91 ± 0.844	23.81 ± 6.296	0.103
∑ PUFA	39.83 ± 6.056	34.48 ± 4.578	40.27 ± 6.154	42.25 ±3.108	37.32 ± 3.521	0.818
PUFA : SFA	0.92 ± 0.204	0.88 ± 0.161	1.02 ± 0.248	1.02 ± 0.127	1.00 ± 0.119	0.969
n6 : n3	0.66^{a} ± 0.023	0.52^{b} ± 0.019	0.49^{b} ± 0.019	0.54^{b} ± 0.024	0.66^{a} ± 0.017	0.010

^{ab}Means with the different letters in a column differ significantly (P<0.05)

Only Leucine, Lysine, Methionine and Valine for the essential amino acid group and Alanine, Proline and Arginine for the non-essential amino acids had significant differences between treatments. It is interesting to note that Control 1 had consistently the lowest concentrations of essential and non-essential (excluding serine) amino acids of all five treatments while a contrasting trend in LM was identified (Table 3.6).

Table 3.6 Amino Acid composition (g/100g) of *H. midae* meat fed five formulated diets over a period of 185 days. All values in the table are given as means ± standard error of the mean (S.E.M.)

Amino Acids	Control 1	Control 2	LN	LM	HM	P-value
Essential						
Isoleucine	1.31 ± 0.163	1.54 ± 0.157	1.59 ± 0.185	1.66 ± 0.086	1.62 ± 0.173	0.573
Leucine	3.79^{a} ± 0.169	4.35^{ab} ± 0.301	4.66^{b} ± 0.242	4.85^{b} ± 0.203	4.75^{b} ± 0.298	0.045
Lysine	2.40^{a} ± 0.198	2.99^{ab} ± 0.236	3.32^{b} ± 0.184	3.52^{b} ± 0.155	3.14^{b} ± 0.248	0.012
Methionine	0.82^{a} ± 0.041	1.04^{bc} ± 0.083	0.97^{ab} ± 0.043	0.91^{ab} ± 0.047	1.16^{bc} ± 0.062	0.006
Phenylalanine	1.54 ± 0.062	1.63 ± 0.103	1.82 ± 0.109	1.87 ± 0.079	1.84 ± 0.138	0.118
Threonine	1.78 ± 0.102	1.91 ± 0.127	2.00 ± 0.153	1.86 ± 0.145	2.02 ± 0.094	0.644
Valine	1.35^{a} ± 0.143	1.53^{ab} ± 0.138	1.73^{abc} ± 0.187	1.99^{c} ± 0.088	1.87^{bc} ± 0.175	0.042
Non-Essential						
Alanine	2.26^{a} ± 0.094	2.51^{ab} ± 0.136	2.79^{bc} ± 0.186	3.02^{c} ± 0.123	2.91^{bc} ± 0.123	0.005
Aspartic acid	5.96 ± 0.242	6.08 ± 0.406	6.47 ± 0.452	6.74 ± 0.355	6.52 ± 0.349	0.553
Cysteine	0.24 ± 0.020	0.30 ± 0.052	0.37 ± 0.038	0.33 ± 0.017	0.31 ± 0.042	0.187
Glutamic acid	6.25 ± 0.654	6.78 ± 0.595	7.47 ± 0.625	7.72 ± 0.627	7.69 ± 0.776	0.451
Glycine	3.39 ± 0.089	3.92 ± 0.081	3.77 ± 0.126	3.57 ± 0.231	3.85 ± 0.134	0.092
Proline	2.52^{a} ± 0.080	2.76^{ab} ± 0.123	2.98^{bc} ± 0.180	3.17^{c} ± 0.132	3.13^{bc} ± 0.112	0.011
Serine	2.77 ± 0.148	2.66 ± 0.210	2.94 ± 0.310	3.41 ± 0.182	3.39 ± 0.168	0.058
Tyrosine	1.67 ± 0.063	1.74 ± 0.125	1.81 ± 0.292	2.06 ± 0.089	2.00 ± 0.150	0.412
Arginine	3.76^{a} ± 0.376	4.07^{ab} ± 0.251	4.34^{abc} ± 0.260	4.93^{bc} ± 0.196	5.19^{c} ± 0.424	0.023
Histidine	0.58 ± 0.039	0.60 ± 0.049	0.64 ± 0.058	0.61 ± 0.045	0.70 ± 0.062	0.559
Hydroxy proline	0.90 ± 0.042	0.92 ± 0.054	0.97 ± 0.097	1.06 ± 0.066	1.07 ± 0.033	0.209

$^{a-c}$Means with the different letters in a column differ significantly (P<0.05)

3.5 Discussion

Obtaining and maintaining a high growth rate (reach market size in the shortest time possible) is essential in order to acquire high production rates, profitability and competitiveness with international markets. Discrepancies in growth rate between experimental studies is not surprising as a number of factors can affect overall growth, such as choice of species, size, age of individuals, stocking density, water quality and temperature as well as the overall duration of the experiment (Stuart & Brown, 1994; Guzmán & Viena, 1998; Naidoo *et al*., 2006). It is therefore difficult to make direct comparisons between studies and such comparisons should be made with caution.

The slowing down of abalone weight gain from December to January indicates either a change in water temperatures, oxygen levels, feeding or sampling strategy. According to Cenni *et al*. (2009) abalone (*H. tuberculata* L.) within the same genetic pool can adopt diverse locomotion strategies, becoming either a 'wanderer' or 'sedentary' individual. Cenni *et al*. (2009) found that the 'wanderer' had significantly better

growth rate than the 'sedentary' abalone while Douros (1987) established that abalone at the bottom of the stack had less accessibility to food, subsequently reducing their overall growth rate. It is therefore hypothesised that each replicate sampled in the current study had abalone displaying both types of locomotion with the wandering abalone positioning themselves nearer the surface of the baskets achieving enhanced feed intake and 'sedentary' individuals located nearer the bottom where food was less abundant. Consequently the present study suggests that the faster growing 'wanderer' abalone were sampled in the first few months of sampling due to ease of access. As the months progressed sampling within baskets became deeper in search of individuals without putty (already sampled). The tapering off of abalone weight gain observed near the end of the study (December) may consequently be partly due to 'wanderer' versus 'sedentary' locomotive strategies where those at the top of the basket grew better and gained more weight due to greater availability of food while those at the bottom had less availability of food resulting in poorer growth.

Though it is common practice to incorporate an acclimatisation period (animals weaned onto new diets/environmental conditions) prior to experiments, it was not conducted in the present study. This may have affected the experimental outcome; Control 1 abalone (consistently fed the commercial Abfeed® diet) displayed the highest growth performance (Ave W_F and % weight gain) and FCR when compared to all other treatments. According to Viana *et al.,* (1996) and Fleming *et al*., (1996) when an acclimatisation period is absent the animals which remain on the pre experimental diet are likely to outperform all other diets as they do not require time to adapt to the new food type. This may have been the case with regard to Control 1 as the abalone remained on the commercial Abfeed® diet during the experiment and adaption time was not necessary. In addition, although lack of acclimatisation is likely to have affected growth performance it is also suggested that Abfeed® may have had a growth advantage over other diets due to enhanced nutritional composition (Britz, 1996a; Britz, 1996b; Dlaza *et al*., 2008). Extensive research has been conducted in the development of Abfeed® to enhance growth and quality of abalone and as a result it is currently the most widely used artificial food for abalone in South Africa (Naidoo *et al.*, 2006; Troell *et al*., 2006; Francis *et al*., 2008; Dlaza *et al*., 2008). Upon closer examination it was apparent that there was a trend that Control 2 performed only marginally worse than Control 1 in a number of the measured growth parameters (Ave W_F, % weight gain, FCR). This suggests that Control 2 (AquaNutro diet) may be a suitable alternative to Abfeed®; however, more research incorporating an acclimatisation period is required to fully assess its potential in enhancing abalone growth.

The lowest growth observed (Ave W_F, % weight gain and % length gain) was in the HCr treatment where abalone were fed a high PUFA diet with added chromium. Although chromium has been shown to increase growth rate of a number of aquatic and terrestrial animals (Page *et al*., 1993; Jain *et al*., 1994; Mooney & Cromwell, 1995; Sahin *et al*., 2002; Sahin *et al*., 2003) the results are not consistent and a number of studies have shown either no growth effect (Bolemann *et al*., 1995; Lindemann *et al*., 1995) or a decrease in the overall growth rate (Page *et al*., 1993). In addition, the quantity of chromium added to the diet can also affect animal growth with variable results between trials (Page *et al*., 1993). These results suggest that a

chromium enhanced diet can have mixed effects and is species, age, size and quantity specific, which highlights the importance of understanding the role of nutrients on a species by species basis rather than making simple inferences across species.

The FCR observed in the present study was between 1.47 and 2.25; although higher than results obtained by Britz (1996a) (1.04-1.15), they were within the ranges described by Britz in a later study (1996b) (0.7-3.4). Both studies by Britz (1996a, 1996b) were performed on juvenile *H midae* (length of 21-29mm and weight of 1.7-4.1g) under controlled (laboratory) conditions (laboratory FCRs vs on-farm FCRs), while differences in experimental procedure between the two studies also existed (protein levels versus protein type), which may account for the variation in results. The optimum FCR is typically ≤1 which signifies that for every gram consumed the abalone will gain ≥1g of weight. Such conversions are infrequent and are more common among juvenile abalone than adults (Britz, 1996b), however, adult FCR can be improved where sufficient trials and research have been executed (as seen in Britz 1996a & b; Britz & Hecht, 1997). Successful optimisation of abalone feed requirements can lower the FCR, subsequently reducing feeding costs and increasing profitability. Although Control 1 was yet again found to out-perform all other treatments Control 2 was observed to have the second best FCR.

The final condition of the abalone in each treatment varied a great deal and it was noted that all treatments decreased in condition over the 158 day period and indicates that these animals increased proportionally more in shell length than weight. No scientific data about the effect of PUFA level and antioxidant additives on shell length growth could be found, therefore more research to determine the effect of these variables on shell growth is suggested.

According to Caballero *et al*. (2002) and Ng *et al*. (2003) PUFA levels had no effect on the growth rate of rainbow trout (*Oncorhynchus mykiss*) and African catfish (*Clarias gariepinus*) respectively. Similar results were found both within the present study (no difference in W_F, % weight gain, SGR_W, SGR_L and CF) and also by Durazo- Beltrán *et al*. (2003) for green abalone (Durazo-Beltrán *et al*., 2003). Abalone fed the low PUFA diets had low FCR, however due to the limited studies examining the effect of lipid types on FCR no appropriate explanation could be found to explain this variation. Overall, these results indicate that increasing PUFA concentrations is not necessarily advantageous for abalone growth and condition of *H. midae*.

Cell membranes containing higher levels of PUFA are more permeable (Olbrich *et al.,* 2000), and prone to increased levels of oxidation (Blokhina *et al.,* 2002; Catalá, 2009) increasing the amount of moisture lost from the cells (Catalá, 2009). It was therefore hypothesized that treatments containing added PUFA's would lose more moisture during post-harvest and *post mortem* and subsequently contains less moisture. However, Control 1 which contained no additionally added PUFA had the lowest moisture content (69.80%) and therefore the higher moisture loss, while treatments HN, LN and Control 2 which contained no additional antioxidants also had lower moisture content (not significant) compared to treatments with added antioxidants. It was shown that the added antioxidants in both the high and low PUFA diets played a vital role in water retention as has been found in previous studies (Cannon *et al.*, 1995; Cannon *et al.*, 1996;

Jensen *et al.*, 1998). Therefore, the addition of antioxidants (green rooibos and chromium) can have a positive effect on the content of water and may increase the overall quality in adult *H midae*.

Protein is an essential nutrient necessary for promoting growth and development (Britz, 1996a & 1996b; Britz & Hecht, 1997) and animals that consume diets containing high levels of protein generally contain high levels of protein and amino acids in their muscle (Britz & Hecht, 1997; Sales *et al*., 2003). All treatments except Control 1 were administered a base diet of NutroScience which contained 10% more protein compared to Control 1 (Abfeed® diet) and as expected Control 1 had lower levels of protein and consequently lower levels of amino acids (except serine). As discussed earlier Control 1 generally had the best growth of all treatments despite the lower protein content which may be due to the lack of acclimatisation compared to the other treatments.

Inconsistencies in fat % content were observed between treatments where the controls, high and low PUFA treatments showed variable levels of fat content with no consistent trend. It was expected that abalone fed the high PUFA diets (sunflower oil) would display higher percentage of PUFA, however this was not the case and in fact the two treatments which contained the low PUFA fat (tallow), had the highest sum of PUFA levels.

3.6 Conclusion

Supplementing the Control 2 diet with antioxidants and high (sunflower oil) and low (tallow) PUFA showed mixed effects. This indicated that PUFA does not play a significant role in abalone growth nor does it modify considerably the muscle composition. An overall comparison of all diets has revealed that Control 1 had the best growth and FCR yet the lowest protein and amino acid levels. This is interesting due to the importance of proteins in growth and development and suggests that other unmeasured variables may have affected the outcome whilst the enhanced growth rate of abalone within Control 1 is likely due to the absence of acclimatisation and requires further investigation. Although Control 1 generally performed the best, Control 2 showed promise on a number of levels (final weight, % weight gain, FCR, SGR_W and CF). Further enhancement of the Control 2 diet with the inclusion of antioxidants may have yielded more positive results and is suggested for future studies.

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CHAPTER 4

DIETARY STRATEGIES FOR LIMITING POST-HARVEST MOISTURE LOSS IN SOUTH AFRICAN ABALONE *HALIOTIS MIDAE*

Abstract

Post-harvest moisture loss has major financial implication on the profitability of abalone farming, however very little has been conducted to examine the factors that influence the extent of moisture loss post-harvest. . In this chapter, various diets were tested to assess their effect on moisture loss at seven different stages from harvest to processing. These stages included a simulated live export process (40 hour duration), the purging period (immersion in clean sea water for 5 days), local transport (from farm to factory), slaughter, 3 hours post mortem, 15 hours post mortem and post cooking (5min boiling). Experimental diets were maintained for six months and included a commercially available abalone diet (Control 1 – Marifeed's Abfeed® K26), a prototype abalone grower diet (Control 2 – NutroScience's AquaNutro Abalone grower diet), a third control diet (Abfeed® K26 and kelp), four high PUFA and four low PUFA diets (containing the NutroScience's AquaNutro as the base diet). Three treatment within the high and low PUFA diet groups were fortified with antioxidants (unfermented green rooibos, mixed tocopherols, chromium) whilst the fourth treatment had no antioxidants added. Results at the simulated live export stage indicated that animals fed the high PUFA diets lost the most moisture (11.3%) compared to the animals fed the low PUFA diets (6.9%) whilst animals in Control 2 lost the least (4.17%). Unfortunately, no data were collected for the third control diet prior to the purging period stage. Nonetheless, the addition of Control 3 presented the opportunity to compare a diet which utilises a combination of both commercial Abfeed® and locally harvested kelp (which is the natural food for abalone) with the other experimental diets. All treatments gained weight during the purging period, but lost weight during local transport from the farm to the factory. Subsequent to slaughter, abalone weight was found to decrease rapidly within the first 3 hours *post mortem* with slight decreases in the following 15 hours whilst negligible moisture was lost due to boiling. Overall it was established that the treatment with high PUFA and mixed tocopherols had the highest moisture uptake during the purging period (8.79%) and the least moisture loss subsequent to cooking (1.89%). This was in contrast to the Control 3 treatment which had the least moisture uptake during purging (1.93%) and the most moisture loss subsequent to cooking (2.33%). These findings can inform industry on best practice to minimising moisture loss and subsequently increase profitability in this growing industry.

Key words: abalone; *Haliotis midae*; moisture loss; processing; feed manipulation; PUFA; antioxidants

4.1 Introduction

Abalone farming is an economically important industry in South Africa in terms of generating foreign income. In 2001, 12 abalone farms, with an estimated value of US$ 12 million, were established along the South African coast (Sales & Britz, 2001). By 2003, this had increased to 18 farms, with a projected production of 527 and 700 tonnes per annum for 2003 and 2004 respectively (Gerber, 2004; Naidoo, 2006). Since 2008

South Africa has grown to become the third largest producer of abalone in the world producing 1036 tonnes (14 farms) of abalone in 2011 with a value of R357 million, representing 94% of the value of the entire marine aquaculture sector within South Africa (DAFF, 2012). The high international demand and restrictions and closures on harvesting wild abalone both within South Africa and beyond (Raemaekers *et al.*, 2011) has ensured prices remain high at between $35 and $45 per kg live weight (Raemaekers *et al*., 2011).

Despite the growth in global abalone aquaculture a number of technical, legislative and physiological issues have been identified. Suboptimal system design, slow growth rates, high juvenile mortalities (reproduction), artificial feed development, inadequate infectious disease control (Britz, 1996b; Bolton *et al.*, 2009; Mouton & Gummow, 2011) and ante and *post mortem* drip loss (Vosloo & Vosloo, 2006; Sales *et al.*, 1999) can severely affect production and profitability within an aquaculture facility. Aerial exposure can significantly affect the water holding capacity of abalone and animals tend to lose moisture (drip loss) due to the high stress experienced when out of their natural environment (salt water) (Vosloo & Vosloo, 2006; van Schalkwyk, 2011). Moisture loss is not restricted to live animals and can also occur *post mortem* on marine gastropods (Olley & Thower, 1977; Sales *et al.*, 1999; Rathgeber *et al*., 1999). Due to high fluctuations in tidal cycles some intertidal gastropods such as mussels, periwinkles and scallops, have developed mechanisms to cope with aerial exposure by retracting the foot muscle and closing the opercular disc, subsequently decreasing drip loss (van Schalkwyk, 2011). However, abalone inhabit the sub tidal zone and have not developed the ability to adapt to aerial exposure, therefore they do not have the ability to protect themselves from *ante mortem* moisture loss (van Schalkwyk, 2011). The *ante* and *post mortem* loss of moisture is a major problem facing abalone farmers as there can be a great deal of weight loss during live export. During this process abalone lose between 4% and 15% of their mass (Vosloo & Vosloo, 2006; Bubner *et al*., 2009), which translates into 40 to 150g per kilogram live weight. As farmers are paid on landed mass, this presents a significant loss of income (Vosloo & Vosloo, 2006).

Due to the high international competition in abalone aquaculture, producing marketable abalone with a lower production cost and increased percentage yield and quality is essential in order to remain competitive and be profitable. In order to increase yield it is necessary to minimise moisture loss during harvesting, handling and transportation. This study will investigate the use of formulated feeds containing different fatty acid profiles and commercially-available antioxidant additives to reduce the amount of moisture lost during a) live transport and b) post-harvest processes in market-sized South African abalone, *Haliotis midae*.

4.2 Materials & methods

4.2.1 Experimental Unit and Animals

The project was conducted at Irvin & Johnson's Abalone Division at Danger Point, Gansbaai, South Africa over a period of 6 months using a combination of flow-through (70%) and recirculatory (30%) systems. The experimental animals were kept in three concrete tanks under normal farm conditions for this period, with a water flow of approximately 20 litre/kg abalone/h, and water temperatures ranging from 15 to 20°C. The

stocking density were 225 animals per basket/replicate with an initial mean weight (±S.E.M) of 57.38g ± 0.293 and mean shell length (±S.E.M) of 64.96mm ± 0.237, respectively.

4.2.2 Treatments

During this 6 month period, the animals were fed 10-11 different diets with 5 replications per diet. The treatments were divided into 3 main feeding categories: four treatments contained high polyunsaturated fatty acids (PUFA) diets (sunflower oil with 65% PUFAs), four treatments contained low PUFA diets (beef tallow with 4% PUFAs) while two-three commercially-available diets were used as controls. The controls consisted of Marifeed's Abfeed® K26 diet (Control 1), NutroScience's AquaNutro diet (Control 2) and a mixture of Marifeed's Abfeed® K26 and kelp based diet (Control 3-only incorporated in phase 2):

- Abfeed® K26 (Stil Street, Lot 2A, New Harbour, Hermanus, Western Cape, South Africa) is the most common artificial food used in South Africa and as standard feeding practice at the experimental site. The feed contains 26% protein, 1.2% fat and 0.9% fiber (Naidoo *et al*., 2006).
- AquaNutro (5 Nywerheid Crescent, Malmesbury Western Cape, South Africa) feed contains 35% protein, 5% fat and 3% fibre and was used as a base diet for all experimental feeds.
- Marifeed's Abfeed® K26 and kelp mixture (The kelp was harvested locally and supplemented the Abfeed® K26 feed). Data was not collected for the third control diet prior to the purging period, since it was not part of the 6 months growth experiment. However the incorporation of Control 3 prior to purging allowed informative comparisons between a mixed Abfeed® and kelp diet, commercial diets and experimental diets.

Each of the high and low PUFA treatments had additives with antioxidant properties integrated into their diet which consisted of DSM's mixed tocopherols with 95% activity (M), Afriplex's 90% unfermented rooibos extract (R) and Alltech's Bio-Chrome with 0.2% activity (Cr) while the fourth treatment in each PUFA group had no additives added (HN and LN).

The eleven treatments/diets will hereon be referred to as Control 1 for Abfeed®, Control 2 for AquaNutro, Control 3 for Abfeed® and kelp mixture (Phase 2 only), LM, LR and LCr for low PUFA diets with additives, LN for low PUFA diets with no additives, HM, HR and HCr for high PUFA diets with additives and HN for high PUFA diets with no additives.

The experiment were conducted in two phases: Phase 1, which examined moisture loss subsequent to live export (40 hours), and Phase 2, which examined moisture loss after the purging period, local transport (1.5 hours), post mortem and post cooking.

4.2.2.1 Phase 1: Simulated Live export moisture loss

The first phase of this experiment was to determine moisture loss during live transport. Due to technical difficulties in replicating a real export process a simulated export process was created in house. Ten animals

per treatment were weighed individually after purging over a 5 day period and subsequently transferred into 100% oxygen filled plastic bags and placed in two separate polystyrene containers. In total 10 bags (n=1 per treatment) were used, each containing 10 animals from each treatment. Ice packs were placed on top of the bags to maintain a low temperature. The polystyrene containers were taped shut and only opened 40 hours later. During this time the polystyrene boxes were exposed to ambient external temperatures for 4 hours and maintained in a cool room (4°C) for the remainder of the experimental period (36 hours). After completing the experiment the animals were weighed and returned to the commercial production system, thereby excluding them from the on-farm experimental system. This protocol prevented the accidental resampling of handled/stressed individuals in the next experimental phase. The moisture loss was calculated by subtracting the body weight of sampled animals after 40 hours from the initial weight and expressed as a percentage of the initial weight.

4.2.2.2 Phase 2: Purging, local transport, post mortem and post cooking associated moisture loss

The four treatments that displayed the lowest live export moisture loss in phase 1 (including Control 1 - Abfeed®) were utilized in phase 2 to determine % moisture loss/gain from purging, local transport of animals between the aquaculture facility and the processing plant (1.5 hours) as well as determining % post mortem and post cooking moisture loss. An extra control group (Control 3) which was not previously examined was also incorporated and sampled as part of phase 2 only and consisted of animals from I&J farm production (not held under experimental conditions) to allow direct comparisons between commercially produced individuals and experimental individuals which were fed formulated and controlled diets. The I&J commercially produced animals (Control 3) were fed a mixed Abfeed® and kelp diet which a number of farms are currently utilising. Though not part of the original experiment the Control 3 animals originated from the same hatching batch and are therefore of the same gene pool, age etc.

Twelve animals from each treatment (n=12) were sampled and weighed (g) at seven different time intervals and weight measurements and calculations were done, as follows:

1. The initial weight ($W_{initial}$) was taken immediately after the animals were removed from the concrete tanks.

2. Subsequent to $W_{initial}$, the animals were placed in mesh purging bags and purged for five days with water temperatures ~15°C and water flow of ~0.1L per second. Purging took place in a flow through system (the water was pumped directly from the sea) and refers to the process of cleaning out the abalone intestine through starvation while immersed in clean seawater. After purging the abalone were weighed. The percentage moisture lost during purging (W_{purge}) was calculated:
    - W_{purge} = (weight after purging - $W_{initial}$) / weight after purging x 100

3. After purging, animals were placed in polystyrene containers and transported (without ice) to Walker Bay Canners in Hermanus, under ambient temperatures. The transport took 1½ hours, where they were weighed again upon arrival. The percentage moisture lost during local transport was calculated:
    - $W_{local\ transport}$ = (arrival weight - W_{purge}) / arrival weight x 100

4. Following local transport, the animals were slaughtered. This consisted of the removal of abalone meat from the shell (shucking), removal of intestines and head and scrubbing the meat with a hand brush until the flesh had a white appearance. Following slaughter, the weight was taken and the percentage yield was calculated:
    - $W_{slaughter}$ = (weight after slaughter – $W_{initial}$) / weight after slaughter x 100

5. After slaughtering, the abalone were placed in individual plastic bags inside of a polystyrene container and transported to Stellenbosch University, again in the boot of the car under ambient conditions. On arrival at Stellenbosch University (3 hours after slaughter), the weight was recorded and the percentage moisture loss calculated:
    - $W_{3h\ post\ mortem}$ = (weight after 3 hours – weight after slaughter) / weight after 3 hours x 100

6. The abalone were stored for a further 15 hours post mortem at 5 ºC. At 18 hours *post mortem* the meat was again weighed and moisture loss calculated:
    - $W_{18h\ post\ mortem}$ = (weight after 18 hours – weight after slaughter) / weight after 18 hours x 100

7. After the *post mortem* (*rigor mortis*) associated moisture loss, six of the 12 animals from each treatment were randomly chosen and analysed to determine the amount of moisture lost during cooking. These animals were individually placed in plastic bags and cooked for 5 minutes in boiling water as suggested by Smit *et al*. (2010). After cooking, the individuals were cooled, patted dry with paper towel, and weighed. The cooking related moisture loss was calculated:
    - $W_{post\ cooking}$ = (weight after cooking – weight after 18 hours) / weight after cooking x 100

4.3 Statistical analysis

All values in tables are given as mean (± standard deviation (SD)). Statistical analyses were performed with SAS Enterprise Guide 4.1 (SAS Institute Inc., USA) and STATISTICA for Windows version 11 (StatSoft. Inc., USA). Differences in the moisture loss of abalone due to live export (40 hours), the purging period, local transport (1.5 hours), post mortem (3 and 15 hours) and post cooking was analysed by one way Analysis of

Variance (ANOVA) with treatment/diet treated as the main effect. Significant differences were assumed if $P<0.05$.

4.4 Results

4.4.1 Phase 1: Simulated Live export moisture loss

At the end of the 40 hour simulated live export trial (phase 1), the Control 2 diet performed the best and had lower moisture loss ($P<0.05$) compared to Control 1 and HN. HN had the highest moisture loss and differed from all the treatments ($P<0.05$), except from Control 1 ($P>0.05$). A summary of the results are presented in Table 4.1 below.

Table 4.1 Summary of the mean percentage live export moisture loss and standard deviation (± SD) between treatments after the 40 hour simulated live transport trial.

Treatment	% Live Export Moisture Loss
Control 1	13.19^{ab} ±7.430
Control 2	4.17^{c} ± 2.362
HCr	9.17^{bc} ± 6.208
HM	6.59^{bc} ± 4.342
HN	18.92^{a} ± 7.217
HR	10.54^{bc} ± 8.374
LCr	8.97^{bc} ± 4.061
LM	5.88^{bc} ± 3.329
LN	4.95^{bc} ± 3.651
LR	7.89^{bc} ± 4.044

$^{a-c}$Means with the different letters in a column differ significantly ($P<0.05$)

Significant differences ($P<0.05$) were notice between the PUFA levels indicating that animals fed the high PUFA diets lost more moisture than the low PUFA diets. A summary of these results are presented in Table 4.2 below.

Table 4.2 Summary of the mean percentage live export moisture loss and standard deviation (± SD) between PUFA levels after the 40 hour simulated live transport trial.

Treatment (40 replicates)	% Live Export Moisture Loss
High PUFA	11.31^{a} ± 7.959
Low PUFA	6.87^{b} ± 3.964

^{ab}Means with the different letters in a column differ significantly ($P<0.05$)

4.4.2 Phase 2: *Ante mortem*, *post mortem* and post cooking moisture loss

After the 5 day purging period, all the treatments increased in weight (Fig.4.1); HM had higher weight gain ($p<0.05$) compared to the production animals (Control 3) but no significant difference was found between Control 1, Control 2, LM, LN and HM. In addition it was also noted that the Control 3 gained the least amount of moisture during this stage of the study when compared to the other treatments but was only significantly different from HM (Table 4.3).

During the 1.5 hour local transport to Walker Bay Canners, all treatments lost weight however; the Control 3 and LM were the only diets that lost more than the moisture they absorbed during the purging phase (lost both the accumulated purged weight and actual body weight).

Immediately after slaughtering and dressing, the animals were weighted again to calculate the percentage yield (% $W_{slaughter}$). According to these calculations animals fed the Control 1 diet had a highest percentage yield ($P<0.05$) compared to all other diets excluding Control 3 ($P>0.05$).

Table 4.3 Summary of descriptive statistics for mean % moisture loss/increase* and standard deviation (±SD).

	% W_{purge}*	% $W_{local\ transport}$	% $W_{slaughter}$	% $W_{3h\ post\ mortem}$	% $W_{18h\ post\ mortem}$	% $W_{post\ cooking}$
Control 1	7.07^{ab} ± 5.31	4.43^{ab} ± 2.70	37.17^{a} ± 2.76	4.12^{a} ± 2.11	5.62^{a} ± 2.37	1.58 ± 0.34
Control 2	5.66^{ab} ± 4.59	5.31^{ab} ± 4.02	31.59^{cd} ± 2.00	2.07^{b} ± 0.44	3.21^{b} ± 0.76	2.15 ± 0.89
Control 3	1.93^{b} ± 3.68	4.01^{b} ± 2.84	34.86^{ab} ± 1.76	3.21^{ab} ± 1.34	4.43^{ab} ± 1.55	2.33 ± 0.80
HM	8.79^{a} ± 4.85	4.14^{ab} ± 3.42	30.29^{d} ± 2.00	3.62^{a} ± 0.86	4.74^{ab} ± 0.96	1.89 ± 0.56
LM	6.33^{ab} ± 5.01	7.97^{a} ± 2.61	33.55^{bc} ± 1.42	1.81^{b} ± 0.64	3.37^{b} ± 1.06	1.83 ± 0.39
LN	6.39^{ab} ± 4.01	4.35^{ab} ± 2.78	32.45^{bcd} ± 2.64	2.05^{b} ± 0.61	3.44^{b} ± 0.85	1.78 ± 0.52

$^{a-d}$Means with the different letters in a column differ significantly ($P<0.05$)
*% W_{purge} – Percentage increase during purging

Eighteen hours *post mortem*, Control 2 lost the least moisture followed by LM and LN, while the Control 1 diet lost the most moisture and differed significantly (P<0.05) from LM, LN and Control 2. There were no significant difference (P>0.05) in moisture loss between Control 2, Control 3, HM, LM and LN 18 hours *post mortem*.

Subsequent to 18 hours *post mortem*, the animals were cooked and moisture loss due to cooking was determined. No significant difference (P>0.05) was found between treatments. Nonetheless it was observed that those individuals that were fed the Control 1 diet lost the least amount of moisture (Table 4.3). The changes in mean abalone weight at different stages of phase 2 are displayed in Fig. 4.1.

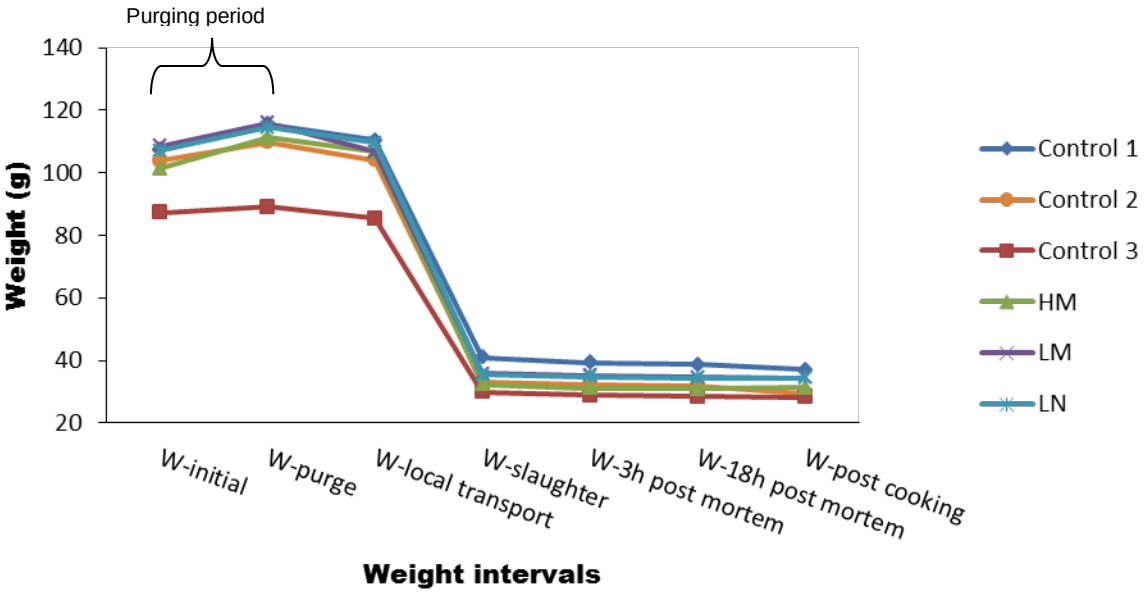

Fig. 4.1 Change in mean weight of abalone at different stages from harvest to cooking.

4.5 Discussion

4.5.1 Phase 1: Live export moisture loss

Moisture loss during live transportation is not restricted to abalone and has been documented for a number of gastropods (James & Olley 1970), and although the factors which affect moisture loss vary between species, it is generally related to evaporation and the production of mucus (Vosloo & Vosloo 2006). The total moisture loss observed in the present study (simulated transport trial) was largely within the same range (4-15%) as described by Vosloo & Vosloo (2006), except for treatment HN (18.92%). Overall it was observed that abalone fed high PUFA diets had a higher percentage of moisture loss (11.31%) than those fed low PUFA diets (6.87%) which may be due to a reduction in cell membrane integrity. Cells containing high levels of PUFA are more susceptible to oxidation (break down of membrane lipids including PUFAs) which compromises the structure and function of the cell membrane resulting in an increased amount of moisture

being lost from the cells (Blokhina *et al.,* 2002; Catalá, 2009). In addition, it is suggested that cell membranes with a high concentration of PUFA are more permeable and weaker than the prototypical monounsaturated membrane (Olbrich *et al.,* 2000). However, the high standard deviation (SD) observed for the high PUFA treatments, indicated that the PUFA level was not the only influencing factor, and that other unknown factors also played a role.

The high additional moisture lost in the HN treatment may be due to the lack of antioxidants present in the diet which makes the cell more prone to oxidation (Ibrahim e*t al.,* 1997; Jenkinson *et al.*, 1999; Eritsland, 2000; Blokhina *et al.,* 2002; Catalá, 2009). Within the high PUFA diets it was apparent that treatment HM had the lowest moisture loss (6.59%) when compared to the other high PUFA treatments (9.17-18.92%) and is likely due to the inclusion of the antioxidant vitamin E (mixed tocopherols). A number of studies have shown a decrease in moisture loss in animals fed diets supplemented with dietary vitamin E (Cannon *et al.*, 1995; Cannon *et al.*, 1996; Jensen *et al.*, 1998) and therefore warrants further investigation

Overall, abalone fed the NutroScience's AquaNutro diet (Control 2), lost the least weight during the 40-hour simulated live export trial. The Control 2 diet had no added PUFA and contained the antioxidant ethoxyguin, that is present in the fish meal used, which may have limited oxidation of the cells and subsequently moisture loss. Little research has been done on abalone moisture loss during live transport, and further experiments are necessary to analyse the effect of dietary manipulation on moisture loss during transport.

4.5.2 Phase 2: *Purging period, local transport, post mortem (3 and 18 hours) and post cooking related moisture loss*

During the 5 day purging period, abalone from all treatments gained weight, which corresponded to results by Francis (2008) on *H. midae*. However, in contrast to Francis's (2008) study, which showed increase in body weight during purging for abalone grown in a flow-through system and decline in a recirculation system, the present study revealed an increase in body weight for all abalone grown in a 70%/30% recirculation/flow-through system. Although Francis (2008) suggested that food retained in the stomach and guts led to the increase in weight gained during the purging period, no such inferences could be made in the present study as stomach contents was not measured. It was hypothesised that stomach and gut contents did not contribute to the increase in weight in the present study as all food digested prior to the purging period would have been excreted prior to the completion of the purging period. Therefore it is assumed that abalone ingested moisture during their purging period and animals which ingested larger quantities of water during purging may have been less stressed during transportation as dehydration was mitigated. This hypothesis has not been previously postulated and may have implications for the abalone industry, in particular the international market. Further research into understanding weight gain during the purging period is required as such uptake can affect the quality and pricing of the end product. Overall, treatment HM had the highest weight gain during purging and shows promise for future investigation and can be beneficial for the live export market.

Moisture lost during local transport in live animals is a common phenomenon which has been previously documented in abalone species and other gastropods (James & Olley, 1970; Vosloo & Vosloo, 2006; Bubner *et al*., 2009; present study). In our study, it was noted that Control 3 lost the least amount of moisture during the short local transportation period and gained the least during purging. It is therefore suggested that a lack of reserve moisture content can affect moisture retention during transport. All treatments lost weight during the short transportation (1.5 hours) from the aquaculture facility to the factory. This can be due to handling stress or temperature shock during storage and transportation since no ice was incorporated into this short transfer period. Although Bubner *et al*. (2009) found that abalone transported with and without ice did not have significantly different moisture loss, their study was conducted over a 35 hour period as opposed to 1.5 hours in the present study. The differences in transport duration between the studies are likely to have affected the variation in results. The initial cooling effect of the ice packs used by Bubner *et al*. (2009) would have subsided over time, leading to an increase in temperature and subsequently stress and water loss in the abalone.

Post mortem moisture loss has been described as the loss of water associated with a decrease in pH (Sales *et al.*, 1999; Huff-Lonergan & Lonergan, 2005) and a decline in the proteins' ability to attract and hold water (Huff-Lonergan & Lonergan, 2005) and can play a significant role in quality aspects of abalone meat, such as texture and tenderness (Sales *et al.*, 1999; Bosworth *et al*., 2007). Subsequent to slaughter, the experimental abalone lost a substantial amount of moisture 3 hours *post mortem*, however this substantial loss was not maintained; only a slight increase in moisture loss was observed between 3 and 18 hours post mortem. From slaughter to 3 hours *post mortem* the abalone were transported from the factory to Stellenbosch University under suboptimal conditions (ambient temperature) which may have significantly increased the quantity of moisture lost in abalone meat, as previous studies shown on terrestrial animal meat (Huff-Lonergan & Lonergan, 2005). According to Sales *et al.* (1999), the pH of abalone meat declines faster under warmer conditions, which may account for the rapid increase in moisture lost during the first 3 hours post mortem and the reduced decrease thereafter. Cool storage is therefore critical in reducing moisture loss during transport. The pH may not be the only variable affecting moisture loss in abalone as degradation of protein muscle increases after slaughter (Olley & Thrower, 1977; Rathgeber *et al*., 1999), subsequently affecting moisture retention in meat.

The negligible moisture loss post cooking was in contrast to a similar study conducted by Sales *et al.* (1999) where 13.14 – 18.85% moisture was lost due to cooking compared to 1.78 – 2.33% in the present study. There are a number of reasons which may account for the differences in moisture loss and reflect variation in methodologies between the two studies. Sales *et al.* (1999) cooked the abalone for 50 minutes at 75°C 7 days *post mortem* while the current study cooked the abalone for 5 minutes at 100°C 18 hours *post mortem* as suggested by Smit *et al*. (2010).

When looking at the overall trends in water uptake and loss throughout the experimental process (Table 4.2 and Fig. 4.1) it was observed that some treatments retained more moisture than others. It is interesting to note that Control 3 gained the least amount of water during purging (1.93%) and lost the most

total moisture subsequent to cooking (8.84%) whilst treatment HM gained the most during purging (8.79%) but lost the least total moisture following cooking (1.98%). These results highlight the importance of the relationship between uptake of moisture during the purging period and moisture loss during transport and processing.

Decreasing moisture loss through manipulation of feed can have significant positive implications for abalone farming as it can help sustain body weight and therefore affect price per individual on arrival at final destinations such as the Asian market. It was interesting to note that the commercial feeds / feeding strategies (Control, 1, 2 and 3) was not optimal for minimising moisture loss. This begs the question: “are current commercial diets the best feed for abalone with regards to moisture retention?” Other diets should be considered and investigated such as diet HM, which outperformed all other treatments in moisture retention.

4.6 Conclusion

Given that abalone price is dependent on weight on arrival at destination rather than weight at departure (farm), optimization of weight retention i.e. moisture content, can have a significant effect on farm revenue and profitability. This study has presented a number of novel findings which have shed light on the understudied topic of moisture loss in abalone. Further experiments and studies are advised to 1) improve our understanding of the physiological reasons behind the increase in moisture content during purging, 2) Identify if different levels of high and low PUFA diets will affect moisture retention and 3) Identify optimal handling and transport conditions to reduce the moisture lost during both short and long haul transportation.

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CHAPTER 5

GENERAL CONCLUSION

Abalone became a high valued commodity globally; which subsequently led to a decrease of the natural stock due to overexploitation and poaching. To meet the high demand for this sought after delicacy as well as for re-stocking purposes, abalone farms have been established globally (USA, Australia, Mexico, New Zealand, Ireland, Iceland and South Africa) (Troell *et al*., 2006). Due to good cooperation between the industry and research facilities in the development of technologies, the abalone industry has grown (Shipton & Britz, 2007). Regardless of the fast growing abalone aquaculture, some technical, legislative and physiological issues have been recognised, which include system design, restricted growth rates (nutrition), high juvenile mortalities (reproduction), artificial feed development, inadequate infectious disease control (Britz, 1996b; Bolton *et al.*, 2009; Mouton & Gummow, 2011) and *ante* and *post mortem* drip loss (Sales *et al.*, 1999; Vosloo & Vosloo, 2006). All of these factors mentioned, can negatively influence the production and profitability within an aquaculture facility. A variety of research projects focusing on different fields, such as nutrition, genetics, reproduction, biotechnology and post-harvest characteristics have been or are currently underway to improve the knowledge and technology for farming of *H. midae* (van Schalkwyk, 2011).

High costs of electricity, feed and labour, together with slow growth rate, is contributing to high production costs of abalone. Therefore, abalone farmers are looking for alternatives, to reduce the production cost, by either ranching abalone in the ocean (no electricity, artificial feed and much less labour), through genetic improvement schemes, production of facility specific feeds on site, or by improving the feed conversion rate and growth through diet manipulation. This in turn can produce new threats.

Since specially formulated commercial abalone feeds are expensive, farmers have begun experimenting with formulated feed on site, however it is crucial that the nutritional requirements of abalone are understood, so that alternative diets and feeding strategies can be utilised which is not always the case with on-site production. An optimum growth rate can be achieved, through an accurate balance of dietary nutrients and accomplishment of the essential nutrients and energy (Smith, 1989; Gomez-Montes *et al*., 2003). Thus, an adequate nutrition and resulting growth of farmed abalone are crucial factors in the successful farming of the South African abalone.

Another major concern experienced by abalone farmers is the high moisture loss (4-15%) during long haul live export. Since farmers are paid on landed mass, the decrease in moisture and subsequently weight results in a decrease in foreign income (Vosloo & Vosloo, 2006; Bubner *et al*., 2009). By optimization of body weight retention (i.e. water content), it can have a positive effect on the farm income, resulting an increasing profit that can lead to further investments and expansion in the industry, subsequently creating jobs and training for the local community. As a result of global competitiveness in abalone aquaculture, it is of upmost importance to produce market size abalone with a lowest possible production cost in the shortest time possible, as well as increase the percentage yield and meat quality.

This project was conducted to obtain more information on the influence of diets containing two different oils (animal and vegetable origin) with low (beef tallow) and high (sunflower oil) polyunsaturated fatty acid levels and various antioxidant additives. The relatively new NutroScience's AquaNutro diet was used as the base diet for the experimental treatments while the commercially available Marifeed's Abfeed® K26 was also included so that the new diets were not simply compared to each other but were also compared to the currently most popular feed on the market.

In the first study (Chapter 3, growth trial), mixed results were obtained from the AquaNutro diet containing oils with high and low PUFA and with added antioxidants. The results suggest that PUFA levels and antioxidants did not play a significant role in growth and the overall abalone meat composition. However, low PUFA showed improved results for final weight and FCR. Although the animals in the present study had a similar growth rate to other experiments, it was difficult to compare results as all the other studies investigated either different levels of the same oil or different oils at the same level while differences in age and species also existed. In addition, no literature is currently available on the effects of oils with different PUFA levels and added antioxidants on growth, condition and meat composition. Overall, the AquaNutro diet showed promise on a number of levels, and is suggested for future studies which may enhance this diet with the inclusion of specific antioxidants.

The literature suggests that the inclusion or exclusion of an acclimatisation period prior to the actual experiment can affect the end results (Fleming *et al*., 1996; Viana *et al.,* 1996). Therefore, it was not surprising that the animals fed on Abfeed® showed better growth from the start (Fig. 3.3 and 3.4), due to the lack of an adaptation period in the present study. This highlights the importance of an acclimatisation period, in order to obtain the most accurate data. Differences in the duration of acclimatisation were found in a number of studies which ranges from 14 to 35 days (Coote *et* al., 1996; Viana *et al.,* 1996; Durazo-Beltan *et al*., 2003; Dlaza *et al*., 2008), and suggest that future research is needed to determine to shortest time necessary for the abalone to get use to the new experimental diets. By determining this, experimental time and costs can be reduced.

The results from the second study (Chapter 4), for the 40 hour simulated live export trial (Phase 1), showed that, PUFA and antioxidants can play a vital role in increasing the body water retention, subsequently reducing moisture loss. It was noticed that the animals fed the low PUFA diets had a significantly lower percentage moisture loss after the export trial as was expected, since it is known from the literature that cell membranes containing high levels of PUFA are more permeable and weaker than the prototypical monounsaturated membrane (Olbrich *et al.,* 2000) and are more prone to oxidation (Blokhina *et al.,* 2002; Catalá, 2009). It was also clear that antioxidants played a vital role in protecting the cell membranes and reducing moisture loss. In the present study, the animals fed the AquaNutro diet (Control 2) which was without any additional additives performed the best by losing the least amount of moisture which means more weight when arriving at the destination. To the author's knowledge, no other studies have determined the effect of different PUFA levels with added antioxidants on the moisture loss of abalone during live transport. These results showed good promise as an alternative feed but require further investigation.

All treatments registered a net weight gain during the five day purging period (as seen in Chapter 4, Table 4.2). Such an increase in weight has not been examined in previous studies and highlights that knowledge gaps still exist in our understanding of abalone physiology.

It was observed that the animals which net gain the most moisture during this purging (treatment HM; Table 4.2) period, showed the least net loss of total moisture after transport and cooking respectively, and showed promise for future use. These findings highlight the importance of the connection between a higher absorption rate of water during purging and better moisture retention during handling, transport and cooking. Although most of the animals are intended for the live export market, the retention of moisture during the live transport is very important, rather than moisture retention during cooking. Subsequent to these findings, it can result in significant positive implications for the abalone industry as it can assist in achieving higher weights and therefore prices per animal on arrival at final destination such as the Asian market. However, more research is needed, since these results could not be compared to other findings, due to no literature could be found in relation to the effect of PUFA and antioxidants during purging and processing of abalone. Our results were compared to animals which were fed a combination of Abfeed® K26 and Kelp (production animals which were from the same genetic pool) and found that the production animals gained the least and lost the most amount of water. It is suggested that PUFA levels and antioxidant can play a significant role, and an alternative diet containing PUFA and antioxidants fed to the animals can help to achieve optimum moisture absorption and minimize moisture loss.

Our study yielded promising results during the growth trial, and positive results regarding moisture loss, an issue experienced by every abalone farmer. Based on the results obtained in this project, a few recommendations can be made for future studies:

- Determining the influence of an acclimatisation period on growth and what the minimum time is for abalone to adapt to the experimental treatments. This will yield more accurate growth rate results, and by determining the minimum time, will reduce the experimental time and costs.
- To further test different oils containing low and high PUFA at different inclusion levels with added antioxidants to evaluate the effect on water retention during handling and transport and quantify the effects of dietary lipid composition on cell membrane composition and permeability.
- Study the physiological reasons behind the increase in moisture content during purging and possible ways to maximize the weight gain.
- Identify optimal handling and transport conditions to reduce the moisture lost during both short and long haul transportation
- Investigate possible ways to improve the randomised procedure when sampling maybe by anaesthetise all the animals per replicate. By following this procedure all the animals will be treated the same, therefore resampling of the same individuals can occur without handling bias. However, it should be taken into consideration that the overall results may be compromised by the stress experienced during this activity.

- Determining the minimum sample size for moisture loss trials in order to obtain the most accurate results. In the present study, the standard deviations (SD) were quite high which may indicate that the sample size was too small, and would recommend a larger sample size within a larger population for future studies.

By optimizing the growth rate, reducing the FCR, increasing water absorption and minimising the moisture loss, abalone farmers can remain competitive and meet the high international market demand. Such practice leads to lower production cost and an increase in turnover, subsequently increasing overall profitability.

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